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Food Research

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The Institute of Food Technologists

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ORGANOLEPTIC RATINGS AND ANALYTICAL DATA FOR WINES ANALYZED INTO ORTHOGONAL FACTORS

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(Manuscript received February 15, 1954)

It has been pointed out by Baker and Amerine *(1)* in a previous paper published in this journal that the organoleptic rating of wines is a complex and difficult procedure which requires extensive training. Also, available data are rather scanty to form a basis for an understanding of organoleptic responses in terms of analytical measurements. In *(1)* data were presented on 17 Riesling and 13 Cabernet wines and discussed in terms of analysis of variance and estimating equations based on selected variables. The equations were not too good and it was felt that different statistical methodology might lead to a better understanding of the obscure relationships between analytical and organoleptic determinations and quality of beverages.

In a complex field where the underlying connections have not been sufficiently investigated so that their logical bases are clear it is recommended by Cattell *(2)* that factor analysis be applied. Factor analysis is a statistical methodology invented and developed primarily to study the human mind. The history and development of this powerful method of investigation are detailed by Thurstone *(4)*, Holzinger and Harman *(3)*, and Cattell *(2)*. Harold Hotelling had much to do with the development of factor analysis and the literature contains many references to his work *(2, 3, 4)*. A complete description of factor analysis cannot be given here. However, the factor analysis of the data presented in *(1)* is given with modifications suitable to the particular type of data.

Thus, the purpose of this paper is to apply the methods of factor analysis to a particular set of analytical and organoleptic data and to indicate the type of conclusions that can be drawn from such an analysis. Comparison is made with analysis of variance of the same data. It is well pointed out by Cattell *(2)* that sometimes analysis of variance and factor analysis can be combined to advantage. Factor analysis applies only to linearly related variables.

The present data are somewhat scanty but are sufficient to indicate the method and suggest certain interesting hypotheses. It is hoped that in the future more extensive data will become available at this experiment station or, perhaps, through the efforts of other workers. In any case the present analysis can be made the basis for more intelligent planning of future experiments.

RIESLING WINES

The data consist of values of 19 analytical variables and the scores of 5 tasters making a total of 24 variables. The starting point for a factor analysis is a 24×24 matrix of correlation coefficients based on 17 values for each variable. These data are presented in detail in *(1)*. A preliminary factorization with rotation to simple structure was carried through for all 24 variables. It was apparent that 2 tasters, B and D, were poor and that pH, total SO_2, aldehyde, tannin, and alkalinity had little or no loading

TABLE 1

Correlation coefficients for 17 selected variables for Riesling wines and estimated communalities (diagonal terms)

Variable	1	2	3	5	6	7	11	12	13	14	15	16	17	19	20	22	24
1. Balling	.76																
2. Alcohol	−.65	.63															
3. Extract	.50	.27	.82														
5. Total acid	.71	−.30	.63	.68													
6. Vol. acid	.07	−.56	.24	.31	.85												
7. Sugar	−.52	.60	.09	−.18	.00	.58											
11. Color	.17	.03	.11	−.12	.24	−.06	.75										
12. Iron	.08	−.12	.24	.43	.72	.06	.49	.79									
13. Copper	−.20	.02	−.24	−.23	−.25	.23	.58	.23	.71								
14. Tot. neut. esters	.04	−.08	.31	.10	.57	−.20	.06	.25	−.07	.64							
15. Vol. neut. esters	.28	−.26	.33	.20	.65	−.54	.45	.04	−.16	.67	.82						
16. Glycerin	−.20	.35	.02	.00	−.45	.24	.28	−.32	.36	−.22	−.30	.71					
17. Ash	.48	.01	.57	.22	.46	.13	.64	.53	.07	.72	.57	−.10	.80				
19. K_2SO_4	.30	−.01	.37	.29	.45	−.04	.58	.47	.10	.55	.48	−.02	.57	.78			
20. A	−.46	.32	−.38	−.40	−.49	.18	−.45	−.43	−.20	−.55	−.52	.81	−.51	−.73	.89		
22. C	−.60	.49	−.21	−.52	−.58	.29	−.53	−.92	−.28	−.54	−.53	.20	−.52	−.66	.75	.94	
24. E	−.43	.50	−.02	−.19	−.48	.15	−.62	−.41	−.24	−.47	−.41	.78	−.54	−.73	.70	.70	.92

TABLE 2

Three factor loadings obtained from Table I and their orthogonal rotation to simple structure

Variable	F_1	V_{o} F_2	F_3		F'_1	λ F'_2	F'_3	F'_1	V_{z} F'_2	F'_3	h^2	h
1. Balling	.64	.11	.38	F_1	−.914	.260	−.311	−.47	.54	−.23	.58	.76
2. Alcohol	−.46	.36	.21	X F_2	−.176	.432	.885	.43	.22	.39	.39	.63
3. Extract	.44	.30	.62	F_3	.363	.864	−.350	−.23	.78	−.09	.67	.82
5. Total acid	.53	.19	.38					−.38	.55	−.13	.47	.68
6. Vol. acid	.73	−.31	−.30					−.72	−.20	−.40	.72	.85
7. Sugar	−.35	.44	−.15					.19	−.03	.55	.34	.58
11. Color	.41	.57	−.26					−.57	.13	.47	.56	.75
12. Iron	.61	.33	−.39					−.76	−.04	.24	.63	.79
13. Copper	−.06	.56	−.43					−.20	−.15	.63	.50	.71
14. Tot. neut. esters	.62	−.16	.09					−.51	.17	−.37	.42	.64
15. Vol. neut. esters	.71	−.36	.19					−.52	.19	−.61	.67	.82
16. Glycerin	−.49	.38	.34					.50	.33	.37	.50	.71
17. Ash	.70	.31	.22					−.68	.51	−.02	.64	.80
19. K_2SO_4	.69	.36	−.04					−.70	.30	.12	.61	.78
20. A	−.84	−.12	.27					.89	−.04	.06	.78	.89
22. C	−.87	−.29	.21					.92	−.17	−.06	.89	.94
24. E	−.77	−.08	.50					.90	.20	−.01	.85	.92

See (2) page 195

on the factor for general quality. A second factorization was carried through on the 17 remaining variables not listed above. These 17 variables included the three best tasters referred to in *(1)* as having been judges at the California State Fair.

The correlation coefficients and estimated communalities are given in Table 1. It was found that 3 factors reduced the residuals to an extent that their values could be accounted for by assuming a random sample based on 17 individuals. If we number the variables of Table 1 in *(1)* from 1 to 19 (omitting volatile acid corrected for SO_2) and the 5 tasters 20 to 24 then the factor loadings are as presented in Table 2. The rotation to simple structure is also indicated in Table 2.

The λ-matrix in Table 2 was determined subject to the following conditions:

a) The F'_1-values for the average loadings for the variables 20 (A), 22 (C), and 24 (E) were made as large as possible since these variables define what we mean by quality in Riesling wines as purely as anything we can imagine.
b) The F'_2 and F'_3 columns were filled in so that the whole rotation defined by λ is orthogonal.

The F'-vectors were extended as indicated by Thurstone *(4)* and plotted on a spherical blackboard. The structure seemed to be as "simple" as it could possibly be. Therefore, no further rotations were made. The loadings on the first factor check very closely for the 24 and 17 variables factorizations.

In the interpretation of Table 2 it is tentatively assumed that loadings less than 0.35 are essentially zero and that values above 0.35 may be significant. Variables showing a negative loading on the first factor, which we shall call quality, can be reflected so that the loading on the reflected variable for the quality factor is positive.

We see that the variables taster A, taster C, taster E, iron, and K_2SO_4 are pure measures of quality except for specific factors [see Cattell *(2)*].

Color, volatile acid, total neutral esters, volatile neutral esters, alcohol, and glycerin have noticeable effects on quality but also they have weightings above 0.35 on factor 3.

Ash, balling, and total acid have some effect on quality and in addition have loadings over 0.35 on factor 2.

Extract, sugar, and copper have insignificant loadings on quality.

These findings are in close agreement with the analysis of variance reported in Table 4 of *(1)* with the possible exception of sugar which was found to distinguish between wines in *(1)* but which here shows an insignificant loading for quality.

The first factor is clearly a quality factor corresponding to Spearman's general intelligence factor in psychology and is influenced by all but three of the 17 variables of Table 1. The second factor is characterized by sizeable loadings for the 4 variables ash, balling, total acid, and extract. The third factor has loadings of more than 0.35, plus or minus, on the 8 variables sugar, copper, color, volatile acid, total neutral esters, volatile neutral esters, alcohol, and glycerin.

CABERNET WINES

The data consist of values of 19 analytical variables and the scores of 5 tasters making a total of 24 variables for 13 Cabernet wines *(1)*.

A preliminary factorization with rotation to simple structure was carried through for all 24 variables. It was apparent that 2 tasters, B and E, were poor and that balling, extract, aldehyde, total neutral esters, glycerine, ash, and K_2SO_4 had little or no effect on the factor for general quality. A second factorization was carried through on the remaining 15 variables not listed above. Two of the same tasters are included as for the Riesling wines but one was dropped and another added in his place. The loadings on the quality factor were very similar for the 24 and 15 variables factorization.

The rotated final matrix corresponding to the second part of Table 2 is given in Table 3. In the interpretation of Table 2 it is assumed that loadings less than 0.40 are essentially zero and that values above 0.40 may be significant. Variables showing a negative loading on the first factor, which we shall call quality, can be reflected so that the loading on the reflected variable for the quality factor is positive.

We see that alcohol and taster A are pure measures of quality except for specific factors.

TABLE 3

Four factor loadings for 13 Cabernet wines based on 15 selected variables orthogonally rotated to simple structure

Variables	F'_1	F'_2	F'_3	F'_4	h
2. Alcohol	.57	−.04	.02	−.29	.64
4. pH	.48	.08	.76	−.08	.91
5. Total acid	−.47	−.53	.06	.32	.78
6. Volatile acid	−.38	−.38	.35	.19	.67
7. Sugar	.34	.06	.69	−.43	.88
8. Total SO_2	−.13	−1.05	−.06	−.40	1.13[1]
10. Tannin	−.34	−.07	−.24	−.18	.46
11. Color	−.31	−.70	−.14	−.09	.78
12. Iron	.27	−.32	−.24	−.59	.76
13. Copper	.09	.30	.37	−.52	.71
15. Vol. neut. esters	−.50	−.39	.17	.55	.86
18. Alkalinity	.41	−.11	.33	.46	.71
20. A	1.00	.23	−.12	−.05	1.03[1]
22. C	.75	−.11	.39	.44	.96
23. D	.86	−.14	−.24	−.41	.99

[1] These values are greater than one due to random errors. See *(3)* page 150.

The quality factor also has loadings above 0.40 on the variables pH, total acid, volatile neutral esters, alkalinity, and tasters C and D.

The second factor has loadings above 0.40 on 3 variables (total acid, SO_2, and color).

The third factor has loadings above 0.40 on pH and sugar.

The fourth factor which is necessarily extracted to bring the residual matrix to a sufficiently low level has loadings over 0.40 on sugar, SO_2, iron, copper, volatile neutral esters, alkalinity, and tasters C and D.

Copper, iron, and SO_2 have little or no effect on the variation in quality. The variables volatile acid, sugar, tannin, and color have loadings between 0.30 and 0.40. It is very possible that if data on more wines were available these loadings might turn out to be significant. It should be remembered that Table 3 is based on 13 wines.

It was necessary to extract 4 factors in the present case as compared to 3 for the Riesling wines. In both cases the final factor seems to depend on 8 variables. The quality factor depends on 11 variables for Riesling wines and possibly only on 8 for Cabernet wines. The second factor for Rieslings depends on 4 variables while correspondingly for Cabernets we find 2 factors that depend on 5 variables.

ESTIMATION OF FACTORS

In this section we will consider the possibility of estimating the quality factor for Riesling wines from a small number of non-organoleptic tests. The methods are explained in more detail by Holzinger and Harman *(3)*.

By inspection the variables alcohol, volatile acid, glycerin, and ash were selected as possibilities for estimating the quality factor, F'_1, of Table 2. The coefficients of the estimating equation are given as solutions of the system of equations:

$$\beta_1 - 0.56\,\beta_2 + 0.35\,\beta_3 + 0.01\,\beta_4 = 0.43$$

$$-0.56\,\beta_1 + \beta_2 - 0.45\,\beta_3 + 0.46\,\beta_4 = -0.72$$

$$0.35\,\beta_1 - 0.45\,\beta_2 + \beta_3 - 0.10\,\beta_4 = 0.50$$

$$0.01\,\beta_1 + 0.46\,\beta_2 - 0.10\,\beta_3 + \beta_4 = -0.68$$

The coefficients of such sets of equations are so uncertain that it may not help to increase the number of betas.

The standard scores of the variables listed above, of taster C and standard scores of the various Riesling wines estimated from the β's are given in Table 4. The correlation between estimated standard scores and the

TABLE 4

Standard scores for alcohol, volatile acid, glycerin, ash, and taster C for 17 Riesling wines with estimated quality scores using the indicated betas

Wine	Alcohol	Volatile acid	Glycerin	Ash	Taster C	Estimated score
130	−1.02	0.26	0.56	−0.09	0.61	−0.08
131	−1.02	0.12	1.12	0.21	−1.14	−0.06
132	1.58	0.16	1.12	0.42	−0.92	0.37
133	−0.80	−0.89	0.25	−1.63	0.39	0.99
134	−1.45	−0.89	0.62	−0.79	−0.26	0.49
135	0.28	−0.55	1.18	−1.07	1.70	1.09
136	1.80	−1.32	1.12	1.00	1.05	0.43
137	−1.04	0.73	0.31	1.63	−0.92	−1.21
138	2.23	−0.65	−0.62	−0.65	1.49	0.82
139	0.93	0.11	−0.68	−0.26	−0.04	0.14
140	−1.45	−0.31	−1.18	−0.70	−0.92	−0.17
141	0.71	−0.22	−0.99	0.44	0.83	−0.31
142	0.28	−0.51	−1.18	0.05	0.39	−0.17
143	−0.15	0.12	−0.68	−0.14	−0.26	−0.16
144	1.80	−0.08	1.12	−1.21	0.83	1.38
145	−3.61	3.22	−1.92	0.63	−1.14	−2.37
146	0.93	0.73	−0.12	2.16	−1.57	−1.19
β	0.2160	−0.2243	0.2683	−0.5522	r = 0.68	

observed standard scores for taster C for the 17 wines is 0.68 which is fairly satisfactory considering that the number of wines is small, that only 4 β's are used, and that taster C cannot be considered a perfect measure of quality.

SUMMARY

Factor analysis has been applied to analytical and organoleptic data on two types of wines presented previously *(1)*. By this means it is possible to replace 24 × 24 matrices of correlation coefficients by factor matrices consisting of three or four columns and 24 or fewer rows.

From such an analysis there emerges a general quality factor plus other factors of less immediate interest. The quality factor is shown to depend rather heavily on certain, perhaps different, variables in each case and to be little affected by other variables. These facts are not apparent from inspection of the correlation matrices or on intuitive grounds.

A method of estimating quality ratings of wines on the basis of non-organoleptic tests is indicated which shows sufficient promise to be investigated more extensively.

It is felt that the present treatment will be very helpful in designing other experiments leading to better understanding of the complex relations between analytical and organoleptic data and "quality" of foods and bev-

erages. It should be noted that the organoleptic responses of tasters are treated simply as another measure of quality and that some tasters were shown to be poor on the basis of conformity to the whole correlation matrix.

ACKNOWLEDGMENT

The somewhat laborious computations were performed by Mrs. Frances R. Jones, statistician.

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THE EFFECT OF CITRIC ACID ON SELECTED EDIBLE STARCHES AND FLOURS[a]

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(Manuscript received June 3, 1954)

The thickening and gelling characteristics of starches and flours are modified by the presence of other ingredients of food products and by variations in food preparation processes. Such variations can cause unexpected results which at times cannot be immediately explained. It is well known that when lemon juice is used in puddings and pie fillings, often the product does not thicken or does not gel. An explanation may be that slight variations in the acid content of the juice prevented the paste from thickening or gelling.

This investigation was designed to establish the effects of increasing amounts of citric acid on the thickening and gelling properties of corn starch, soft wheat starch, hard wheat starch, soft wheat flour, and hard wheat flour. Measurements were made of the changes in viscosity during heating of the acidified pastes, and of their viscosity and gelling characteristics after cooling. Observations were made also of the appearance and size of the starch granules under the microscope.

EXPERIMENTAL METHODS

Two wheat flours[c] and a corn starch[d] were obtained commercially in lots sufficient for the entire study. The flours were a bleached soft wheat flour grown and milled in New York State and a bleached hard wheat flour of a patent grade. The wheat starches were prepared in the laboratory by washing them from stiff doughs of the flours.

Pastes were prepared in an amylograph[e] using 450 ml. of dilute citric acid solution for each test. To make the starch pastes, 29 g. of corn starch, and 33 g. of the wheat starches were added as experiments showed that these amounts produced gels with $21 \pm 1\%$ sag. For the flour pastes, 45-g. portions of each of the 2 wheat flours were used because this amount gave a hot paste viscosity not exceeding 700 Brabender units throughout the acid range studied. Concentrations of citric acid were selected in the range 0.0025 N to 0.5000 N which produced wide variations in the viscosity of the pastes. The normalities of the acid used, it should be noted, are somewhat lower than those reported for lemon juice of similar pH.

The effects of citric acid concentration on thickening are shown in Figures 2 and 3.

The viscosities of pastes prepared in the foregoing manner were recorded on amylograms during the progress of heating to 95°C. (Figure 1). Gel rigidity was measured using the method of Cox and Higby *(2)* and as modified by Hester *(4)*. For this measurement, portions of the hot pastes were poured into jelly glasses fitted with metal collars so that the glasses could be filled above the tops. The jelly glasses were covered

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[a] This article is based on a thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of requirements for the degree of Master of Science.

[b] Present address—Food Processing Laboratory, Iowa State College, Ames, Iowa.

[c] Co-operative Grange League Federation Exchange, Inc., Ithaca, New York.

[d] American Maize Products Company, Roby, Indiana.

[e] Brabender Corporation, Rochelle Park, New Jersey.

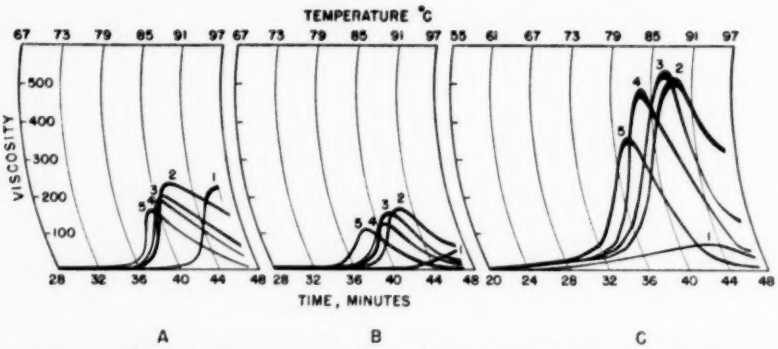

Figure 1. Amylograms showing the effect of heat and increasing citric acid concentrations on the viscosity of starch and flour pastes.

A. Corn starch.
1.—no citric acid.
2.—0.010 N. citric acid.
3.—0.050 N. citric acid.
4.—0.100 N. citric acid.
5.—0.300 N. citric acid.

B. Soft wheat starch.
1.—no citric acid.
2.—0.010 N. citric acid.
3.—0.050 N. citric acid.
4.—0.100 N. citric acid.
5.—0.300 N. citric acid.

C. Soft wheat flour.
1.—no citric acid.
2.—0.010 N. citric acid.
3.—0.050 N. citric acid.
4.—0.100 N. citric acid.
5.—0.500 N. citric acid.

and stored overnight at 30°C. The gel was sliced off level with the top of the glass and the rigidity measured with the Exchange Ridgelimeter.[1] The results were expressed as per cent sag. The consistency of non-gelling pastes was compared by measuring their area of spread by the method of Grawemeyer and Pfund *(3)*.

A 5-ml. aliquot of each acid solution and of each hot paste at the termination of heating was diluted with distilled water to 125 ml., and the pH of this solution was determined using the Beekman pH meter.

The amount of soluble starch and its amylose content in some of the starch pastes were estimated to determine whether or not these values were correlated with gel properties. The method of precipitation of the soluble starch was essentially that of Schoch and French *(7)*. An aliquot of each paste was washed and centrifuged and the supernatant liquid decanted 4 times using a total of 200 ml. of distilled water. Three hundred ml. of commercial methanol were added to a 50-ml. aliquot of the pooled supernatant liquid. The precipitate of soluble starch was collected in a tared, sintered, Pyrex crucible, washed with methanol, dried over calcium chloride for 24 hours in a vacuum desiccator and weighed. The percentage of water-soluble material was calculated on the basis of the weight of air-dried starch in the paste.

A colorimetric titration method was used to determine the relative amount of amylose in the soluble material. The material was dissolved and diluted according to the directions of Kerr *(5)*. The methods of titration and measurement of the increase in the blue color were those developed by Hester *(4)* from the work of Kerr *(5, 6)* and Bates *et al. (1)*. The percentage of amylose in the soluble starch was calculated from the following formula:

$$\text{Per cent amylose} = \frac{\text{g. iodine absorbed}}{(\text{g. starch in the cuvette})\ (X)},$$

where X represents the iodine affinity of the pure amylose isolated from either corn starch or wheat starch. X for the corn starch paste = 18.7% *(1)*; X for the wheat

[1] California Fruit Growers Exchange, Ontario, California.

starch pastes = 19.9% *(7)*. Since the values for X were calculated from clear solutions containing completely soluble material, and since it was difficult to obtain clear solutions of the material in this study because of the high degree of fragmentation which took place when the pastes contained citric acid, these values for X may not be the true values for these samples. Although this error was recognized, no correction was applied to the formula.

For microscopic examination, samples of each hot paste were removed (a) immediately after the amylograms showed a rise in viscosity, (b) immediately after maximum viscosity had been reached, and (c) at the termination of heating. Each sample was dispersed in 25 ml. of hot distilled water and stained with a solution of 0.2% iodine dissolved in 2.0% potassium iodide. A drop of each suspension was examined using a phase microscope adjusted to dark contrast and a magnification of 430×. A photomicrograph was made of the field containing the largest granule observed on each slide. Diameters of these largest granules were estimated from the photomicrograph using a calibrated eyepiece micrometer scale which was photographed with each field, and the volumes were estimated assuming a spherical shape for the granule. Relative volumes of the swollen granules were calculated using as reference the volume of the largest granule of the same starch observed similarly in a cold water suspension.

RESULTS AND DISCUSSION

The difference between the pH of any acid solution and the pH of the pastes prepared with that solution was never greater than 0.05 pH units. Evidently, neither the starches nor the flours affected the acidity of the pastes. As the concentration of citric acid was increased, the pH decreased from 5.95 for those solutions and pastes which contained no added acid to 3.20 for those which were 0.5 N with respect to citric acid.

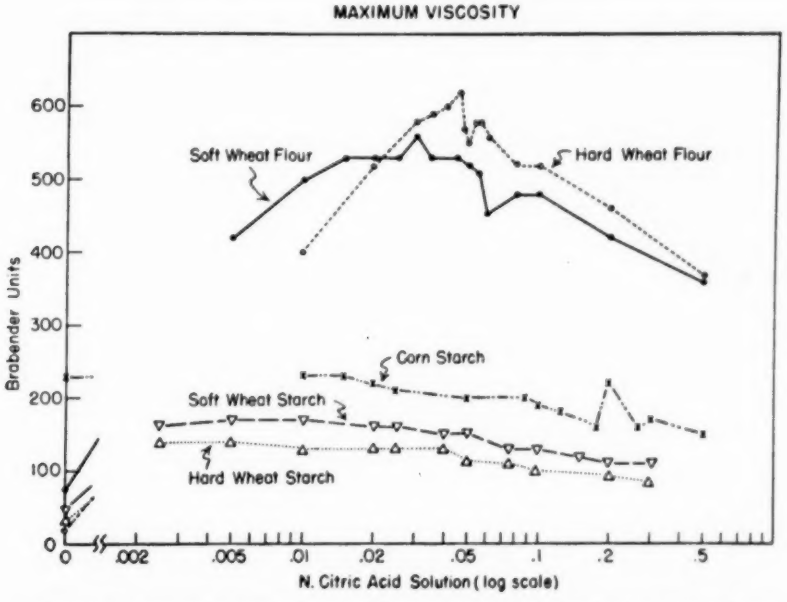

Figure 2. Changes in maximum viscosities of starch and flour pastes with changes in citric acid concentration.

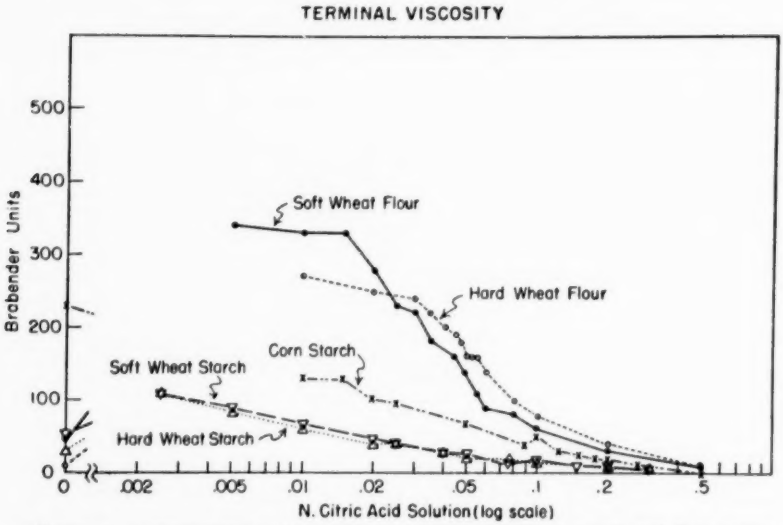

Figure 3. A comparison of the changes in the viscosities of starch and flour pastes at the termination of heating (95°C.) with changes in the citric acid concentration.

Amylograms of selected starch and flour pastes are presented in Figure 1. In Figure 2 the maximum viscosity values of all pastes are plotted against the concentration of citric acid, and in Figure 3 the terminal viscosity values are similarly presented. For the corn starch and wheat starch pastes, increasing the acid concentration lowered the temperature of the initial increase in viscosity and lowered the temperature and magnitude of the maximum viscosity. Thinning of the pastes after they had attained maximum viscosity took place wth the lowest amount of acid; the amount of thinning increased as the acid concentration was increased.

Amylograms for the hard wheat starch were almost identical with those for the soft wheat starch; amylograms for the 2 flours were alike. The shape of the curve A 1 (Figure 1) indicates that the corn starch paste containing no acid probably reached maximum viscosity at 95°C., the termination of the record, for the curve was almost flat during the last minute of heating. The curve for the wheat starch paste containing no acid showed no sign of flattening during the heating period. Amylograms of this same starch heated in water to 95°C. and held at that temperature for 15 minutes showed that maximum viscosity was reached when the temperature of the paste first reached 95°C. Pastes containing corn starch thickened more rapidly than those containing wheat starch. Maximum and terminal viscosities of the corn starch pastes were greater than those of the corresponding wheat starch pastes.

Photomicrographs of the starch granules from some of the soft wheat starch pastes at maximum viscosity and at termination of heating are shown in Figure 4. The pastes were the same ones from which amylograms 1, 2, and 5 in Figure 1 were obtained. Changes in appearance of the corn starch and hard wheat starch granules corresponded to those of the soft wheat

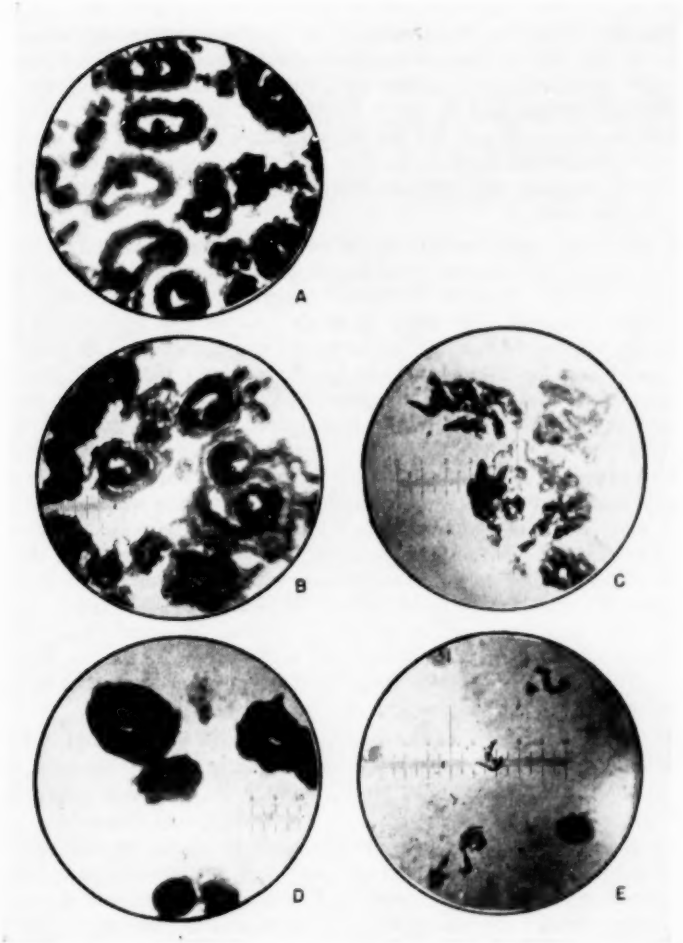

Figure 4. Photomicrographs of soft wheat starch granules at different stages of heating and with different concentrations of citric acid.

A. No acid, maximum viscosity (50 B. U., 95° C.).
B. Citric acid 0.010 N, maximum viscosity (170 B. U., 88.75° C.).
C. Citric acid 0.010 N, terminal viscosity (70 B. U., 95° C.).
D. Citric acid 0.300 N, maximum viscosity (110 B. U., 83.5° C.).
E. Citric acid 0.300 N, terminal viscosity (10 B. U., 95° C.).

starch granules. In general, the swollen corn starch granules were larger than corresponding wheat starch granules. At maximum paste viscosity, the starch granules heated in low concentrations of acid showed less implosion than when heated in water. The greater rigidity of the unimploded granules might account for the greater maximum viscosity of the starch

pastes in the lowest concentration of acid when compared with the maximum viscosity of pastes containing no acid. There seemed to be a slight decrease in the size of the swollen granules at maximum paste viscosity in the acid solutions more concentrated than 0.010 N. In all pastes made with acid only fragments of the granules could be seen when heating of the pastes was discontinued. As the concentration of the acid was increased, the granule fragments became smaller and more difficult to observe. The thinning of the paste is probably accounted for by the fragmentation of the starch granules.

For the flour pastes, increasing the concentration of citric acid did not affect the transition temperature, but the temperature at which the pastes attained maximum viscosity decreased progressively as the acid concentration was increased. The effect of citric acid on the viscosity of wheat flour pastes was very great, as can be seen from Figures 1C, 2, and 3. At all stages of heating, the viscosity of the flour pastes was much greater in the presence of acid than in the presence of water. Maximum viscosity decreased as the acid concentration was increased beyond 0.030 N for the soft wheat flour and 0.040 N for the hard wheat flour. Terminal viscosity decreased after the first addition of acid. Judging from microscopic observation, it was concluded that the starch granules in the wheat flour pastes swelled more and suffered less from fragmentation than did the granules in the wheat starch pastes. It is possible that some component of the flour formed additions or complexes with some of the acid and thus protected the starch granules in the flour pastes. It is also possible that, since the greatest difference between the wheat flour and its starch was probably the protein in the flour, the addition of and change in the concentration of the citric acid had an effect upon the flour proteins such that they contributed greatly to the viscosity of the flour pastes.

The ultimate thinning of the wheat flour pastes may be due in part to the action of the acid on the starch granules and in part to diastatic action. Both of these influences would tend to make the granules susceptible to fragmentation.

The effect of increasing concentrations of citric acid on the rigidity of the starch and flour gels is illustrated in Figure 5. Decrease in the rigidity of the starch gels as the concentration of citric acid was increased may have been caused by the increased fragmentation of the granules. When the curves for terminal viscosity of the hot pastes (Figure 3) are compared with those for per cent sag of the gels formed by the cooled pastes (Figure 5), it will be seen that the relationship between viscosity and gel strength is a complex one and that it is different for the three starches. In the corn starch pastes the relationship appears to be the least complex. In the flour pastes there seems to be little relationship between the viscosity of the hot pastes as they were poured into the jelly glasses and the sag of the gels 24 hours later. Indeed the hard wheat flour pastes formed such soft gels that only the most firm of them could be measured in the ridgelimeter. It is evident that the components of flour other than starch and the concentration of citric acid are playing very important roles in the viscosity of the pastes and the rigidity of the gels.

For the determination of soluble starch, the pastes made with water

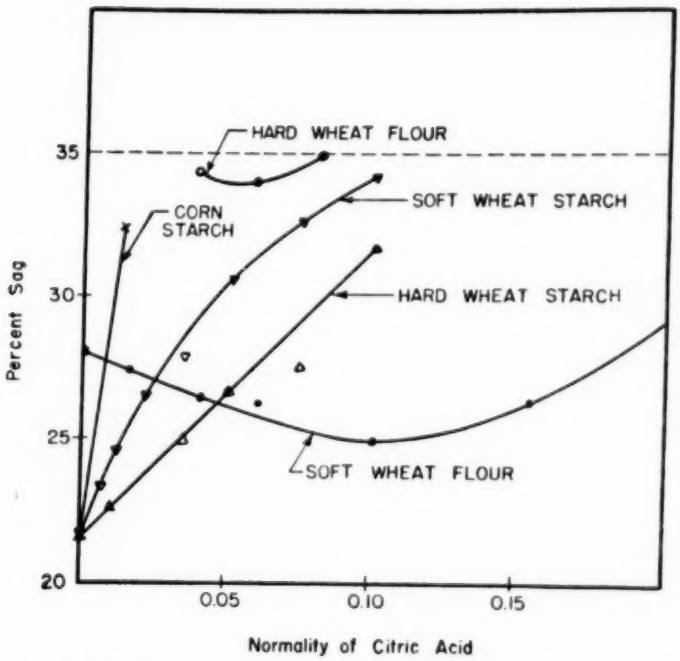

Figure 5. The effect of increasing concentrations of citric acid on the per cent sag of starch and flour gels.

alone and the pastes of the 3 starches which gave the gels of the greatest measurable sag were selected. For the soft and hard wheat starches, pastes of intermediate citric acid concentration which gave gels of the identical sag were also selected. The amount of soluble starch and the amylose content of the soluble material leached from the granules during heating in water and in selected citric acid solutions was greater in the acid solutions, especially in those of the higher concentrations (Table 1). The supernatant liquid from all the pastes was somewhat turbid and the turbidity was greater in the presence of citric acid, especially the higher concentrations. It is possible that the turbidity was due to granule fragments which could not be settled by centrifugation. Consequently, some of the material which was precipitated as soluble starch may have been minute granule fragments. The increase in the fragmentation of granules with increasing concentrations of acid in the pastes may, however, account for only part of the increase in the amount of soluble material. It must also be recognized that when starch granules have been ruptured, more material may be solubilized.

The iodine uptake of the soluble corn starch was slightly greater and that of the soft and hard wheat starches was somewhat less with acid than with water alone. As the concentration of citric acid was increased, the iodine uptake by the soluble wheat starches decreased. However, because

TABLE 1

The effect of citric acid on the quantity and composition of the water-soluble starch leached from corn starch and wheat starches

Kind and amount of starch		Citric acid concentration	Soluble starch	Iodine uptake by soluble starch	Amylose in the soluble starch	
	g.	*N*	%	%	%	*g.*
Corn	29.0	0.000	9.6	8.6	46	0.29
		0.010	15.5	9.5	51	0.51
Soft wheat	33.0	0.000	18.5	13.0	65	0.88
		0.025	49.8	7.1	36	1.30
		0.100	69.1	5.8	29	1.47
Hard wheat	33.0	0.000	19.6	7.6	38	0.55
		0.050	56.0	7.0	36	1.43
		0.100	70.0	6.5	32	1.67

of the greater amount of soluble starch in the pastes containing acid, the total amount of amylose in solution increased with acid for all 3 starches. If the amounts of soluble starch were progressively overestimated because of greater fragmentation, the calculated total amounts of amylose probably were progressively higher than the true values, even though the proportion of amylose in the soluble starch diminished.

Considering all the above data it is obvious that fragmentation of the starch granule as well as the type of starch were the factors most affecting rigidity of the gels.

SUMMARY

Effects of increasing amounts of citric acid on the thickening and gelling properties of corn starch, two wheat starches, and two wheat flours were studied.

Results of this work show that increasing concentrations of citric acid lowered the gelatinization temperature of the starches, increased the fragmentation of the granules, reduced the maximum viscosity, and increased the amount of subsequent thinning of the pastes. In wheat flour pastes, addition of small amounts of citric acid caused a marked increase in maximum viscosity, and, as the citric acid concentration was further increased, the maximum viscosity of the pastes decreased and subsequent thinning was increased. On the basis of microscopic observations, thinning of the starch pastes made with acid was attributed to increased fragmentation of the granules. The starch granules in the wheat flour pastes made with citric acid swelled more, and suffered less from fragmentation, than those in the wheat starch pastes.

Starch gels became softer with increasing quantities of citric acid. Flour gels stiffened with small amounts of the acid and softened with larger amounts. Because of the increased fragmentation of the starch granules with increase in the concentrations of acid, conclusions relating changes in the amount of amylose to the changes in the rigidity of the gels could not be drawn.

The effect of citric acid on the wheat flour gels could not be explained

on the basis of the effect of the acid on the starch alone. Further investigation is necessary to determine the effect of the acid on the components of wheat flour other than starch in relation to the thickening and gelling characteristics of flour pastes.

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THE EFFECT OF FREEZER STORAGE TEMPERATURES ON CAKE QUALITY AND ON THE CARBON DIOXIDE CONTENT OF CAKE BATTERS*

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In recent years several investigators have been concerned with factors affecting the keeping quality of frozen cakes and batters. Among the factors that have been reported to influence keeping quality are freezer storage temperature *(3, 5)* and the ingredients used, particularly the type of baking powder *(5, 8)*.

Graul and Lowe *(3)* reported that cakes baked from frozen batters were reduced in volume and had deteriorated noticeably in flavor, aroma and color after 2 months of storage at 0°F. However, they found products baked from similar batters and stored at –10°F. were not significantly different from freshly baked cakes.

Mackey, Jones, and Dunn *(5)* reported that "cakes baked from batters stored at 0°F. up to 3 months were markedly smaller in volume and more compact in texture than cakes baked from freshly prepared batters of the same kind." Cakes baked from batters stored at –30°F. had good volume up to 6 months, but tended to show irregularities in texture and flavor as storage time progressed, depending on the type of fat used. They reported that cakes baked from tartrate batters stored at 0°F. showed more noticeable volume decreases than those baked from sulfate-phosphate batters stored at the same temperature.

Zaehringer and Mayfield *(8)* compared the effects of sulfate-phosphate and tartrate powders in cakes and batters stored at –10°F. They reported that, when the batters were frozen and stored in cartons, the sulfate-phosphate batters produced better quality cakes up to 4 months of freezer storage. Cakes baked from sulfate-phosphate batters were similar in quality to freshly baked cakes, but those baked from tartrate batters had decreased in quality because of lower volume, compressibility, and palatability values. When batters were frozen and stored in baking pans, significant volume decreases occurred with both types of baking powder.

Presumably some of the volume and quality changes reported in these studies could have been due to loss of potential leavening in the form of carbon dioxide during freezer storage of the batters. Each type of baking powder differs in the rate at which carbon dioxide is liberated when in contact with moisture. Therefore, the present study was designed to give more information about conditions conducive to the retention of quality in cake batters during freezer storage. The primary objectives were to determine: *(a)* how much carbon dioxide is lost from cake batters when

* Published with the approval of the Vice-Director of the Tennessee Agricultural Experiment Station.

frozen and stored at different temperatures; *(b)* to what extent the carbon dioxide loss is dependent upon the type of baking powder used; and *(c)* the relationship of carbon dioxide loss to quality changes in cakes baked from frozen batters.

PROCEDURE

Four series of shortened cakes, frozen in the batter state, were tested after 2, 4, 8, and 12 weeks of freezer storage. Batters were prepared with each of the three types of baking powder commonly used for home baking: sulfate-phosphate, phosphate, and tartrate. They were frozen and stored under the following temperature conditions: (a) frozen and stored at -20°F.; (b) frozen at -20°F. and stored at 0°F.; (c) frozen and stored at 0°F. During the period of the investigation the temperature in the -20°F. room fluctuated from -16°F. to -20°F. The temperature in the zero room fluctuated from 0°F. to -3°F.

Preparation of the batters. The formula for the cake batters was adapted from one by Fowler and West *(2)*. Adjustments were made in mixing time, method of mixing, amount of baking powders *(4)*, and baking temperatures until cakes of equivalent quality were obtained with each type of baking powder before freezing.[b]

The flour, sugar, shortening, and baking powder for the first 2 series of cakes were purchased in a single lot. For the last 2 series these ingredients were purchased in separate lots. Milk was purchased daily from the University Creamery. Eggs were obtained weekly from the University Poultry Farm.

Batters were mixed in a Model N-50 Kitchen Aid electric mixer. Immediately after mixing, seven 265-g. portions of batter were weighed into 6-inch square, tin, baking pans. One pan of batter was baked immediately and tested as freshly baked cake. The other 6 pans of batter were put into freezer storage. The remainder was used for determining the carbon dioxide content of the freshly prepared batter.

Packaging, freezing, and storing batters. All pans of batter for freezer storage were placed in cellophane bags, heat sealed, and overwrapped with stockinette. Two pans of batter were stored under each of the 3 temperature conditions, one to be baked for quality evaluation and volume measurement, the other for determining the carbon dioxide content of the batter after freezer storage.

Baking cakes. The same baking procedure was used for freshly mixed batters and for those that had been held in freezer storage. Frozen batters were thawed to 59°F. before baking. Batters containing sulfate-phosphate baking powder were baked at 300° F. for 10 minutes and 375°F. for 20 minutes. Phosphate batters were baked at 325°F. for 10 minutes and 375°F. for 20 minutes. Tartrate batters were baked at 350°F. for 10 minutes and 375°F. for 20 minutes.

TESTING CAKES AND BATTERS

Measuring carbon dioxide content of batters. The A.A.C.C. *(1)* gasometric method for determining the carbon dioxide content of baking powders with a Chittick apparatus was adapted to the determination of the carbon dioxide content of the cake batters. Determinations were made in duplicate on freshly mixed batters and on thawed batters, as soon as the temperature reached 59°F. A 75-g. sample of batter was used for each determination. The carbon dioxide was released from the batter by adding 40 ml. of 1:3 H_2SO_4. The batter was stirred magnetically during a 20-minute collection period. If there was a temperature rise in the reaction flask during the determination, the volume of gas collected was corrected for the expansion of air in the flask before calculating the carbon dioxide content of the batter.

Testing baked cakes. Quality and volume measurements were made on cakes baked from freshly prepared batters and on those baked after freezer storage. The volume index was measured by polar planimeter according to the method of Platt and Kratz *(7)*, and the quality of the cakes was evaluated by sensory difference tests. A panel of 3 to 4

[b] The formula and method of mixing may be obtained from the authors upon request.

experienced judges graded the cakes using a 100-point scoring system. The characteristics graded and maximum values allowed for each were: shape, 10; volume, 15; crust, 5; grain, 10; color of crumb, 5; texture, 15; odor, 10; taste, 30. Three samples arranged in random order on a tray were scored at one time. They represented the 3 storage conditions for cakes containing one type of baking powder.

RESULTS

Effect of freezer storage temperatures on the carbon dioxide content of cake batters. The carbon dioxide content of fresh and frozen cake batters containing different types of baking powder is shown in Table 1. The per cent changes are compared graphically in Figure 1. All batters lost some carbon dioxide during freezer storage. With each

TABLE 1

Carbon dioxide content of cake batters before and after freezer storage

Type of baking powder used in batter	Freezer storage time	Carbon dioxide content of batters			
		Freshly prepared	After freezer storage		
			Frozen and stored at −20°F.	Frozen at −20°F. stored at 0°F.	Frozen and stored at 0°F.
	weeks	%	%	%	%
Sulfate-phosphate	2	.071 ± .004	.060 ± .003	.052 ± .003	.054 ± .003
	4	.064 ± .003	.053 ± .002	.045 ± .003	.045 ± .001
	8	.068 ± .003	.051 ± .005	.037 ± .002	.037 ± .005
	12	.069 ± .003[1]	.053 ± .001[1]	.033 ± .004[1]	.035 ± .005[1]
Phosphate	2	.065 ± .002	.063 ± .003	.058 ± .004	.057 ± .003
	4	.065 ± .004	.060 ± .003	.052 ± .005	.051 ± .003
	8	.066 ± .003	.056 ± .006	.038 ± .003	.041 ± .003
	12	.065 ± .003	.058 ± .002	.032 ± .004	.033 ± .005
Tartrate	2	.053 ± .003	.046 ± .004	.043 ± .002	.042 ± .002
	4	.058 ± .004	.048 ± .002	.036 ± .001	.036 ± .002
	8	.055 ± .002	.043 ± .004	.025 ± .003	.026 ± .003
	12	.051 ± .004	.040 ± .005	.017 ± .003	.017 ± .003

[1] Average of duplicate determinations on three replications. All other figures are averages of duplicate determinations on four replications.

type of baking powder there was a greater loss of gas from batters stored at 0°F. than from those stored at −20°F. After 8 and 12 weeks of freezer storage this decrease in carbon dioxide content of the batters held at 0°F. was significant. In several tests the carbon dioxide loss from batters held in zero storage exceeded 50%, while at −20°F. none of the batters lost more than 25% of the original carbon dioxide. At both storage temperatures the loss of carbon dioxide from each type of batter tended to increase progressively with prolonged storage. Freezing at −20°F. prior to storage at 0°F. produced no consistent or marked improvement in retention of carbon dioxide. In most cases the carbon dioxide content of batters frozen at −20°F. before storing at 0°F. was about the same as that of the batters frozen and stored at zero.

Effect of type of baking powder on the carbon dioxide content of cake batters. Phosphate batters stored at −20°F. retained a higher per cent of carbon dioxide than the other batters stored at this temperature. The sulfate-phosphate and tartrate batters tended to lose carbon dioxide at about the same rate in −20°F. storage but a little faster than phosphate batters.

During the first 4 weeks of freezer storage at 0°F. phosphate batters retained slightly more carbon dioxide than either sulfate-phosphate or tartrate batters. However, beyond 4 weeks of freezer storage, the sulfate-phosphate and phosphate batters tended to lose carbon dioxide at about the same rate with maximum losses of about 50% at the 12-week test period. Tartrate batters lost approximately two-thirds of the initial carbon dioxide during 12 weeks in zero storage.

Cake volume changes. Volume indices for freshly baked cakes and cakes baked from frozen batters are shown in Table 2. The per cent changes are compared graphically

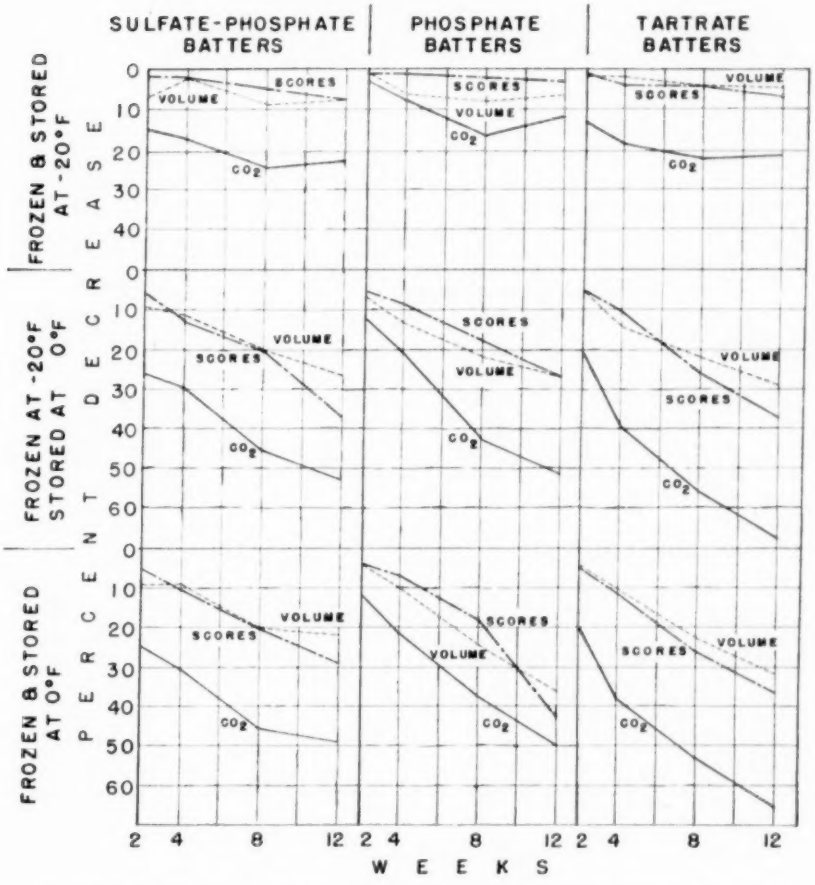

Figure 1. Per cent decrease in carbon dioxide content of frozen batters and in volume indices and quality scores of cakes baked from these batters.

in Figure 1. With all types of baking powder there was no significant decrease in the volume index of cakes baked from batters stored at −20°F. At this storage temperature, the decrease in volume index did not exceed 8% for any baking powder at any test period.

Greater decreases in volume occurred in cakes baked from batters stored more than 2 weeks at 0°F. The decreases in volume index were significant for cakes baked from: sulfate-phosphate batters frozen at −20°F. and stored 12 weeks at 0°F.; all phosphate batters stored 12 weeks at 0°F.; and all tartrate batters stored 8 and 12 weeks at 0°F.

Quality changes. Quality scores are shown in Table 3, and the per cent changes are compared graphically in Figure 1. Cakes baked from batters stored at −20°F. were approximately equivalent in quality to freshly baked cakes, as indicated by the small percentage decreases in total scores. Batters stored for 2 weeks at 0°F. produced cakes of similar high quality, scoring only 4% to 7% lower than freshly baked cakes. After this period, the deterioration in the quality of cakes baked from batters stored at 0°F.

TABLE 2

Volume indices of freshly baked cakes and cakes baked from frozen batters

Type of baking powder used in batter	Freezer storage time	Freshly baked	Baked after freezer storage		
			Frozen and stored at −20°F.	Frozen at −20°F. stored at 0°F.	Frozen and stored at 0°F.
	weeks	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Sulfate-phosphate	2	45.5 ± 3.1	42.7 ± 2.7	41.3 ± 4.5	41.6 ± 1.9
	4	42.6 ± 2.3	41.9 ± 1.6	37.4 ± 3.0	38.6 ± 3.2
	8	44.3 ± 2.6	40.9 ± 1.3	35.5 ± 1.6	35.3 ± 3.3
	12	43.4 ± 1.9[1]	40.3 ± 1.7[1]	31.8 ± 2.7[1]	33.8 ± 3.0[1]
Phosphate	2	47.9 ± 2.3	47.9 ± 1.0	44.7 ± 1.2	45.6 ± 1.2
	4	48.2 ± 0.7	45.7 ± 2.3	42.1 ± 1.0	43.3 ± 1.8
	8	49.1 ± 1.7	45.6 ± 0.5	38.4 ± 1.2	37.0 ± 4.6
	12	48.3 ± 1.7	45.4 ± 1.7	35.5 ± 2.5	30.5 ± 2.5
Tartrate	2	45.5 ± 1.0	45.1 ± 1.4	43.2 ± 1.5	43.4 ± 1.3
	4	47.1 ± 2.1	46.3 ± 1.6	40.5 ± 2.1	42.0 ± 1.5
	8	45.9 ± 1.2	44.4 ± 0.6	35.9 ± 1.9	35.3 ± 3.3
	12	45.0 ± 0.9	42.9 ± 0.8	31.8 ± 2.7	30.7 ± 2.1

[1] Average of three replications. All other figures are averages of four replications.

TABLE 3

Quality scores of freshly baked cakes and cakes baked from frozen batters

Type of baking powder used in batter	Freezer storage time	Freshly baked	Baked after freezer storage		
			Frozen and stored at −20°F.	Frozen at −20°F. stored at 0°F.	Frozen and stored at 0°F.
	weeks				
Sulfate-phosphate	2	95.7 ± 1.8	94.0 ± 4.1	89.3 ± 6.2	90.1 ± 5.4
	4	92.9 ± 1.0	91.6 ± 1.2	80.1 ± 5.5	83.3 ± 2.7
	8	94.4 ± 1.8	90.0 ± 2.1	75.0 ± 4.4	74.5 ± 8.7
	12	94.9 ± 0.8[1]	88.8 ± 1.5[1]	60.0 ± 6.3[1]	68.2 ± 6.8[1]
Phosphate	2	95.2 ± 2.6	94.5 ± 1.0	90.2 ± 2.9	91.5 ± 2.1
	4	94.6 ± 2.6	94.5 ± 0.5	86.2 ± 3.7	88.0 ± 3.0
	8	94.0 ± 1.7	92.9 ± 2.6	76.8 ± 5.3	75.9 ± 9.6
	12	95.7 ± 0.1	93.1 ± 1.0	69.2 ± 7.3	55.1 ± 5.6
Tartrate	2	95.2 ± 1.4	94.1 ± 2.7	90.6 ± 2.4	89.8 ± 1.8
	4	96.5 ± 1.0	93.7 ± 2.0	86.0 ± 2.5	85.4 ± 4.2
	8	93.9 ± 2.9	91.0 ± 2.4	69.4 ± 6.2	68.8 ± 7.9
	12	94.8 ± 0.8	89.2 ± 2.3	59.8 ± 6.7	59.5 ± 7.9

[1] Average of three replications. All other figures are averages of four replications.

was more pronounced. Cakes baked from each type of batter stored at this temperature for 8 and 12 weeks were heavy and tough in texture, compact in grain, had thickened cell walls, and occasional soggy, waxy streaks, extending horizontally across the layer. The reduction in the total quality scores for these cakes was due largely to the decreases in scores for volume, grain, and texture. No improvement in the quality of the cakes was obtained by quick freezing at −20°F. prior to zero storage. The comparatively high standard deviations for the cakes of poorer quality may indicate inconsistency on the part of the judges when grading a product that deviates considerably from a standard. There is also a possibility that the rate of deterioration in quality varied more during the longer storage periods.

DISCUSSION

Analysis of the data indicates that the quality of cakes baked from frozen batters was affected more by freezer storage temperatures than by the type of baking powder (Figure 1). Greater carbon dioxide retention, larger volume and higher quality scores on all cakes baked from batters stored at –20°F. indicated that less deterioration occurred in these batters than in similar batters stored at 0°F. No batters were stored longer than 12 weeks. During this period no noticeable impairment of flavor occurred in cakes baked from frozen batters stored at either temperature. Only slight changes in texture and grain were observed in cakes baked from batters stored at –20°F. Mackey, Jones, and Dunn *(5)* reported that a coarse, irregular texture and off-flavor developed in cakes containing either lard or vegetable shortening when stored for 6 months at –30°F.

The protection to quality provided by the lower temperature may be related to the freezing point of the batters. Some preliminary work indicated that these cake batters crystallized between 2°F. and 0°F. Therefore, batters stored in the zero room were held at approximately their crystallization points. They were about the texture of hard ice cream when removed from storage. The rapid loss of carbon dioxide from batters stored at 0°F. may indicate that this temperature was not sufficiently below the freezing point of the batters to give protection against carbon dioxide losses. No additional protection against carbon dioxide losses was evident when batters were frozen at –20°F. prior to zero storage.

In the present study all batters were stored in baking pans. There is a possibility that the carbon dioxide losses and quality deterioration might have been reduced if the batters had been stored in cartons. Owen and Van Duyne *(6)* obtained better quality cakes when frozen batters containing sulfate-phosphate baking powder were stored in cartons. Zaehringer and Mayfield *(8)* obtained better quality cakes when batters containing tartrate or sulfate-phosphate baking powder were stored in cartons, than when stored in baking pans; the tartrate batters were inferior to the sulfate-phosphate batters, however.

There seemed to be a direct relationship between carbon dioxide losses from the stored batters, and volume and quality deterioration in cakes baked from these batters. On a percentage basis carbon dioxide losses were greater at each test period, but tended to parallel the volume and quality changes occurring in the baked cakes (Figure 1). Presumably, therefore, some of the deterioration in cakes baked from frozen batters in this study was due to loss of carbon dioxide during freezer storage of the batters. No attempt was made to determine whether air incorporated into the batters during mixing was retained during freezer storage.

SUMMARY

Four series of shortened cake batters were tested after 2, 4, 8, and 12 weeks of freezer storage. Batters were prepared with three types of baking powder: sulfate-phosphate, phosphate, and tartrate. Freezing and storing conditions were: frozen and stored at –20°F.; frozen at –20°F. and stored at 0°F.; and frozen and stored at 0°F.

There were no marked differences in the quality of cakes obtained with the different types of baking powder, under similar storage conditions. The quality and volume of cakes baked from frozen batters did not deteriorate noticeably during 12 weeks of storage at –20°F. Marked deterioration occurred in cakes baked from batters containing each type of baking powder when stored at 0°F. for more than 2 to 4 weeks. Greater losses of carbon dioxide occurred when batters were stored at 0°F. than when stored at –20°F. Quality deterioration tended to parallel carbon dioxide losses. The greater quality deterioration and carbon dioxide losses during 0°F. storage may have been due to the fact that this storage temperature was not appreciably below the freezing point of the batters.

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CONTRIBUTION TO THE CARBOHYDRATE CHEMISTRY OF MAPLE SAP AND SIRUP[a]

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Reports in the literature dealing with the carbohydrates of maple sap have been mainly limited to sources of the sugar, relationships of tree structures and functions to sugar formation, causes of sap flow and other factors influencing the sugar content. These indicate sap to contain only a few sugars of which sucrose is highly preponderant. Jones *(10)* reported hexoses, as represented by the reduction of Fehling's solution, in small quantities ranging from 0.003% to 0.081% with an average for 50 samples of 0.021%. Holgate *(8)* reported 0.0005 to 0.0025% invert sugar in sterile sap collected from the same tap hole as a season progressed. Bois and Nadeau *(1)* have reported the presence of cellobiose and suggest that this compound may be responsible for the small reducing power of sap.

This paper reports investigations, made as part of a study of color and flavor development during the processing of sap to sirup, which indicate that the carbohydrates consist of a relatively intricate mixture of oligosaccharides.

MATERIALS AND METHODS

Materials. The maple sap for these experiments was obtained from hard maple trees *(Acer saccharum)* in a sterile condition by means of a modification *(11)* of the technique of Holgate *(8)*. All samples of sap were checked for sterility by plating on nutrient agar. Any samples producing more than one colony per ml. were rejected. Only sap from runs collected in the 12 hours prior to processing were used. Strict sterility is necessary to prevent contamination of the sap with fermentation products.

Ion exchange methods. The fresh sterile sap was deionized by exchange resins *(12)* to obtain a neutral or carbohydrate fraction. To remove basic ions, 1700 ml. of cation resin (Dowex 50[c]) in a spherical form was employed in the [H^+] ion cycle in a glass ion-exchange column, 2 inches in diameter and 60 inches long, equipped for down flow exhaustion and regeneration and for back-washing at 50% bed expansion. A flow rate of 0.26 ml. per minute per ml. of resin was used throughout the experiments. For regeneration, 4 bed-volumes of 2N HCl were used, and the column was washed with distilled water until the effluent was free of chloride ions.

The acidic ions were removed with 1700 ml. of an amine-type anion exchange resin (Duolite A-4) in the [OH^-] ion cycle. The column dimensions and flow rate were the same as for the cation column. For regeneration, 4 bed-volumes of 1N NH_4OH were used, and the column was washed with distilled water until the effluent was acid to phenolphthalein.

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[c] Mention of commercial products does not imply endorsement by the Department of Agriculture over others of similar nature not mentioned.

Chromatographic methods. The choice of methods and the order of their use were dictated by the fact that the exceedingly large excess of sucrose over the total of all other carbohydrates made it difficult to obtain fractions of the different sugars which were completely free of sucrose. The major portion of the sucrose was removed by a preliminary resolution on the carbon column of Whistler and Durso *(14)*. Paper chromatograms were used both for isolation of fractions and for identification experiments. The solvents used were 1-propanol, ethyl acetate, water [7-1-2] *(6)*, 1-butanol, pyridine, water [3-1-1.5] *(9)* and 1-butanol, ethanol, water [10-1-2] *(16)*. Sprays employed included benzidine-citric acid in butanol *(15)* as a general reagent for all sugars, naptho-resorcinol-phosphoric acid in 95% ethanol *(3)* as a ketose detecting reagent, and triphenyltetrazolium chloride *(15)* as a reducing sugar reagent. The large-scale partition method on cellulose powder, suggested by Gross and Albon *(6)* was used for the separation and study of the monosaccharides. A paper ionographic method using a laboratory constructed apparatus and conditions and procedures modified from several techniques in the literature *(4)* were used to effect resolution of several sugars appearing as chromatographic entities.

Enzyme and acid hydrolysis. An ultramicro technique for the enzymatic hydrolysis of sugars prior to papergram analysis *(13)* was employed in most cases. No buffer was necessary with the invertase[d] and invertase plus melibiase[e] enzyme preparations used at the sugar concentrations under investigation. Equal volumes of sugar solution [5-10 mg./ml.] and enzyme solution [10 mg./ml.] were employed. Mild acid hydrolyses were made using 0.05 N HCl at room temperature for one hour. Strong acid hydrolyses consisted of heating at 100°C. in 1N HCl for one hour.

PROCEDURE AND RESULTS

Experimental. Twenty gallons of sterile sap (2% solids) were freed of ions by passing the sap solution through the cation column at the stated flow rate and the effluent was passed directly into the anion column by means of a glass tubing connection. The 2 columns were washed in series with distilled water until the final effluent was free of sugars. The eluate and washings from the 2 columns were concentrated to 66.5% solids in vacuo at a temperature less than 35°C. as fast as delivered from the columns. This concentrate was designated the *neutral fraction*.

Monosaccharides. The original sterile maple sap, free of fermentation products, after concentration in vacuo, showed a slight reducing power by Schoorls alkaline copper method *(2)* which was equivalent to about 0.002% invert sugar on a sap basis after correcting for the amount of reduction due to the amount of sucrose present. The water eluate from a carbon column resolution showed a varying amount of reducing power depending upon the flow rate of an individual column. This amount of reduction, when calculated as percentage of invert in the sap, was always more than the 0.002% of reducing substance, as invert sugar, found in the original sap. This indicated hydrolysis during the carbon column resolution.

To determine the presence or absence of pentoses or hexoses, 20 g. of deionized neutral fraction was placed on a large scale cellulose column (7 cm. by 37 cm.) and resolved with 2-propanol, 1-butanol, water [7-1-2] according to Gross and Albon *(6)*. Fractions of 125 ml. each, taken by means of an automatic fraction cutter, were evaporated to dryness and taken up in 0.1 ml. of water. Papergram analysis of each successive fraction failed to detect the presence of monosaccharides. As a further check, all of these fractions (from the first to those showing sucrose) were combined, evaporated to dryness and dissolved in 0.1 ml. of water. Papergram analysis of this composite fraction also failed to show the presence of monosacharides. A mixture of equal parts of glucose and fructose when added, to the extent of 0.005% of the sucrose present (0.0001% on a sap basis), to the neutral fraction was easily detected by papergram analysis after combining all of the fractions up to sucrose from a cellulose column, evaporation to dryness and solution in 0.1 ml. of water.

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[d] Invertase, melibiase free from top fermenting yeast, Wallerstein Laboratory, gelatin vehicle.

[e] Invertase plus melibiase from fermenting yeast, Nutritional Biochemicals Corporation, gelatin vehicle.

Oligosaccharides. To each of 5 carbon-celite columns (3.4 cm. by 17 cm.), determined by analysis to have sucrose capacities varying from 2.33 g. to 2.45 g., was added 25 g. of the deionized neutral fraction (16.63 g. solids) diluted to about 20% solids with water. Five columns were used to obtain the sugars in sufficient quantities for further purification and study. Each column was eluted first with 5 liters of water, then with 4 liters of 5% ethanol. Those eluates were discarded since they contained, by papergram analysis, only mono- and disaccharides (mainly the excess of sucrose due to the overloading of the columns). The oligosaccharides were eluted completely from each of the columns with 1.5 liters of 50% ethanol. The 5 eluates were combined and concentrated in vacuo (less than 35°C.) to a small volume and freeze-dried (weight = 0.4187 g.). Papergrams indicated appreciable quantities of sucrose still to be present. A 20% water solution of this freeze-dried material was prepared and refractionated on a new carbon column by eluting successively with 1.8 liters of water, 3 liters of 5% ethanol and 2 liters of 50% ethanol. The water and 5% ethanol eluates were again discarded.

The 50% ethanol eluate was concentrated in vacuo and freeze-dried to give a residue weighing 197.7 mg. Papergram analysis indicated the presence of 5 oligosaccharide fractions, one of which was shown to be sucrose. A portion of this material (128.9 mg.) was dissolved in water and streaked on a large filter paper sheet (Whatman No. 1, 14 × 22½ inches) as a narrow band (5 mm.) and the papergram was developed with the 1-butanol, pyridine, water reagent for 24 hours. The zone positions were detected by the usual means and excised. The oligosaccharide fraction was eluted from each of the resulting paper strips with water and the extracts were dried by freeze-drying. These dried extracts were chromatographed on paper using 2 other solvents and appeared to be homogeneous.

These 5 fractions and their hydrolytic products are described in Table 1. Zone No. 1 was shown to be a trace of sucrose.

TABLE 1

Description of oligosaccharides isolated by the carbon technique and by filter paper chromatography from 83.2 g. (dry wt.) of neutral fraction

Zone No.	Rs[1]	Wt. of extracted material, mg.	% of neutral fraction	Products of complete hydrolysis with HCl[2]	Products of mild HCl hydrolysis[3] or invertase
1	1.00	8.6	0.0069	Glucose + fructose[5]	Glucose + fructose
2	0.80	17.7	0.014	Glucose + fructose	Glucose + fructose
3	0.51	20.3	0.016[4]	Glucose + galactose + fructose	Unk. oligosaccharide + fructose
4	0.20	19.3	0.015	.....	.....
5	0.00	38.8	0.031	Glucose + galactose + fructose	.....

[1] Mobility compared to sucrose in 1-butanol pyridine-water.
[2] 1 N HCl at 100°C. for 1 hr.
[3] 0.05 N HCl at 25°C. for 1 hr.
[4] Equivalent to less than 0.0005% of the original sap.
[5] Monosaccharides identified by comparison with known compounds.

The oligosaccharide from Zone No. 3 was hydrolyzed with invertase, streaked on filter paper and chromatographed with 1-butanol, pyridine, water. A new oligosaccharide and fructose were resolved. This oligosaccharide was eluted from the paper. Chromatography in two other solvents gave no further separation and so indicated homogeneity. Preliminary paper ionographic experiments *(4)* on the original trisaccharide [0.05 M. borate, pH 9.2, 7 v./cm., 3.6-4.2 m.a.] indicated two major and possibly two very minor components. Analysis by the same method of the oligosaccharide fraction derived from the original by invertase indicated the presence of two compounds, one of which had the same mobility as melibiose, the only disaccharide run as a known.

The results of the action of the different hydrolyzing reagents on this derived oligosaccharide fraction and the unhydrolyzed trisaccharide are given in Figure 1.

A sample of the original extract from Zone No. 3, weighing approximately 200 μg., was hydrolyzed with invertase and chromatographed on paper. The disaccharide spot and the fructose spot were excised and eluted with water. The disaccharide sample

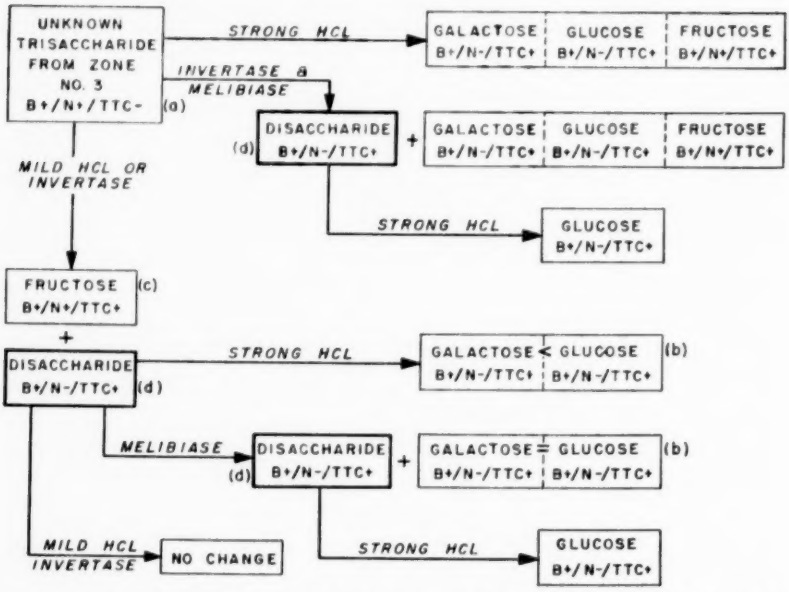

Figure 1. The enzymic and acid hydrolytic products of the trisaccharides isolated from zone No. 3.

(a) Indicator reagents: B = Benzidine-citric acid; N = Naphthoresorcinolphosphoric acid; TTC = Triphenyltetrazolium chloride.

(b) Semi-quantitative measurements of glucose and galactose made by visual comparison of the benzidine developed spots with standards of equal parts of glucose and galactose run simultaneously on the chromatogram.

(c) Estimated by micro reduction to be in a ratio of one mole of fructose to one mole of disaccharide.

(d) Each system of blocks indicates spots observed on a papergram as a result of hydrolysis of the starting material in question. Those components not isolated are separated by dotted lines. The heavily outlined blocks indicate this material was isolated from the hydrolytic products by paper chromatography for the purpose of further study.

was hydrolyzed by refluxing at 100°C. in 1N HCl for one hour and neutralized with NaOH. Shaffer and Somogyi microdetermination *(2)* of the sugars showed 70 μg. of fructose and 135 μg. of sugars as glucose from the disaccharide indicating one mole of fructose per mole of disaccharide or one mole of fructose per mole of original trisaccharide.

Rechromatography of the original extract from Zone No. 3 on carbon indicated the unhydrolyzed compounds in the mixture to be trisaccharides since they were eluted with 15% ethanol. The oligosaccharide fraction, produced by the action of invertase on the original extract from Zone No. 3 was eluted from another carbon column with 5% ethanol, indicating that only disaccharides were present.

DISCUSSION

The formation of monosaccharides during resolution of the neutral fraction of sterile sap by use of carbon columns has not been explained. Slight acidity of the carbon combined with the enormous surface furnished by the carbon particles may be the explanation but for the fact that solu-

tions of pure sucrose passed through the same column did not yield any increased amount of invert sugar over that present in the sucrose before chromatographing. A further possibility is the hydrolysis of some oligosaccharide which is more labile than sucrose. As identification of the several sugars progresses, their stability on the carbon column will be studied.

When extremely mild conditions were employed (partition chromatography on cellulose powder) no monosaccharides could be detected in the sterile maple sap (0.0001% of invert sugar added to the sap could be easily detected). Sap collected under non-sterile conditions but processed within 36 hours showed a detectable amount of glucose and fructose. As microbial contamination increased, proportionally more glucose and fructose were detected confirming the results of Holgate *(8)* and Hayward *(7)*.

The chromatographic and hydrolytic data indicate that sterile sap contains, in addition to sucrose, at least 5 other oligosaccharides. The experience with the original extract from Zone No. 3, which was apparently homogeneous by chromatographic criteria, but which was shown to be a mixture of two trisaccharides by hydrolytic and paper ionographic techniques, lends doubt to the homogeneity of the 3 other fractions. Therefore, there may be more than 5 oligosaccharides in addition to sucrose. Results similar to these were reported by Gross *(5)* in his study of the paper electrophoresis of oligosaccharides synthesized from sucrose by yeast invertase.

Examination of the results reported in Figure 1 for the sugars isolated from Zone No. 3 shows the following:

a. The trisaccharides produce galactose, glucose and fructose upon strong acid hydrolysis.

b. Invertase plus melibiase hydrolysis produces galactose, glucose and fructose plus a disaccharide which yields only glucose upon hydrolysis with strong acid; therefore, one of the trisaccharides must have two adjoining glucose units.

c. Mild acid or invertase hydrolysis causes the formation of a disaccharide mixture and fructose which by analysis were shown to be in a 1:1 ratio. Therefore, each trisaccharide unit must contain a fructose unit. Hydrolysis with invertase indicates that each trisaccharide must have a sucrose unit with a terminal fructose.

d. Melibiase hydrolyzes one part of the disaccharide mixture resulting from the mild acid hydrolysis to yield galactose and glucose in equal proportions but does not hydrolyze the other disaccharide. Since melibiase is specific for an α-galactoside linkage, one trisaccharide must consist of melibiose plus fructose and since it contains a sucrose linkage it can be concluded that it is probably raffinose or a closely related compound. The disaccharide remaining after melibiase hydrolysis yields only glucose when hydrolyzed with strong acid. Therefore, the second trisaccharide must consist of a diglucose disaccharide and fructose and since it contains a sucrose linkage it is a glucosyl sucrose.

R_x data on the diglucose disaccharide [0.41 in 1-propanol, ethyl acetate, water], when compared to maltose [0.69] and cellobiose [0.61], eliminated it as being one of these. Further work to characterize the linkages for final identification of this sugar is in progress.

The characterization of the materials isolated from Zones numbered 2, 4 and 5 will be the subject of future papers.

SUMMARY

Maple sap, if collected under sterile conditions, contains less than 0.0001% by weight of monosaccharides. It has been demonstrated to contain at least five oligosaccharides in addition to sucrose. One fraction isolated by paper chromatography and apparently homogeneous by chromatographic standards was shown to be a mixture of two trisaccharides which lends credence to the possibility that other fractions may be mixtures. One of the two trisaccharides of the mixed fraction has been established to be raffinose or a closely related compound and the second to be a glucosyl sucrose.

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FACTORS RELATING TO THE FORMATION OF A FAT-RING ON HOMOGENIZED MILK[a]

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Since the advent of every-other-day and three-times-per-week milk delivery to the home and five- or six-day plant operation, many serious problems have emerged in regard to the keeping and physical qualities of milk. Not the least of these is the appearance of a "fat-ring" which appears at the surface and is attached to the sides of bottles of homogenized milk after

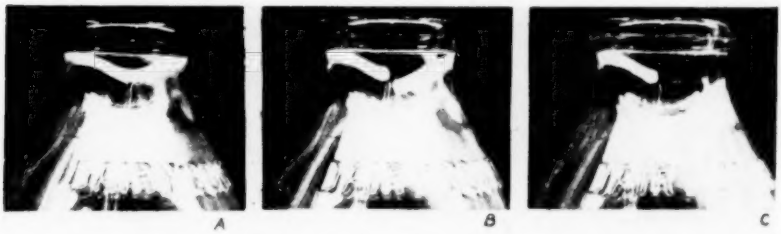

Figure 1. Appearance of the fat-ring on homogenized milk at successive intervals following pouring. A — One minute. B — Two minutes. C. —Four minutes.

some of the milk has been poured from the bottle (Figure 1). Many milk bottling plants throughout the United States have been troubled with this defect and have sought corrective measures. Since many of the factors associated with fat-ring formation are not known, a study of the cause of this defect seems timely.

EXPERIMENTAL

Milk processing and handling procedures were studied as well as variations in the composition of milk thought to have some relationship to the formation of the fat-ring on homogenized milk.

Influence of heat shocking and extended storage. Homogenization indices, as defined by the United States Public Health Service Milk Ordinance and Code *(6)*, were made on 196 quart bottles of homogenized milk obtained from 4 different sources and stored at 40°F. for a period of one week. Daily, 2 quarts of the milk were removed from storage and allowed to stand at room temperature for a period of 30 min., then, returned to the refrigerator. On the following day, the top 100 ml. of these 2 samples, plus similar portions of 2 control samples, which had been inverted for 30 min. but had not been exposed at room temperature, were tested for fat. The 100-ml. portions were obtained by simple decantation. The fat tests were compared to those from the respective remaining portions.

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The heat-shocking treatment favored fat-globule rising up to 48 hr. storage. However, when milk became 72 hr. old, the previous heat-shocking treatment did not result in an increase in the homogenization index over that of the control samples. These observations, recorded graphically in Figure 2, indicate that any fat particles which contribute to the formation of a fat-ring will have risen to the top of homogenized milk within 72 hr. following homogenization. The data show also that the tendency toward fat-ring formation is not influenced materially by the heat-shocking treatment to which the samples were subjected.

Influence of homogenization pressures. Pooled milk was pasteurized at 145°F. for 30 min. Portions of this milk were homogenized at 130°F. at pressures ranging from 2000 + 500 to 6000 + 500 p.s.i. (2-stage). Data representing those trials are given in

TABLE 1

The influence of homogenization pressures upon the degree of fat-ring formation on homogenized milk

Homogenization pressure (2-stage)	Fat ring formation		
	Percentage of fat in milk		
	3.6	3.7	4.1
(p.s.i.)	*(degree)*	*(degree)*	*(degree)*
2000 + 500	1	1	4
3000 + 500	0	1	2
4000 + 500	0	0	2
5000 + 500	0	0	1
6000 + 500	0	0	0

Legend: no fat ring = 0
slight fat ring = 1
distinct fat ring = 2
prominent fat ring = 3
cream plug = 4

Table 1. Fat-ring formation accompanied low homogenization pressures and decreased with an increasing total pressure of homogenization. This was particularly true in the case of the samples with the highest fat concentrations.

Influence of homogenization temperatures. Pooled milk was heated to 190°F. and held at this temperature for 30 minutes. A portion was cooled immediately to 40°F. and stored for 24 hours. The remainder of the milk was divided into 7 lots and homogenized immediately at definite temperatures (190°, 170°, 150°, 110°, 90°, and 70°F.) after which the samples were cooled to 60°F., bottled and stored at 40°F. for one week. The homogenization pressures used were 3000 + 500 p.s.i. (2-stage). After 24-hr. storage at 40°F. the portion of milk which was cooled immediately following pasteurization was warmed to the previously selected homogenization temperatures and homogenized. The homogenized samples were then cooled to 60°F., bottled and stored for one week at 40°F. Following one week of storage at 40°F., all samples were examined for fat-ring formation. The fat-ring material was recovered from the surface of the milk and analyzed for fat, total solids, and ash content.

The intensity and nature of the fat-ring on the bottles of homogenized milk were examined by means of the unaided eye and expressed on a relative basis. The composition of the fat-ring material which formed on milk homogenized at various temperatures, together with the degree of fat-ring formation are shown in Table 2. These data indicate that as the concentration of solids-not-fat per gram of fat, and more particularly, as the concentration of ash per gram of fat in the fat-ring material increases the extent of the fat-ring formation is lessened.

Data in Table 3 show the influence of the homogenization temperature on the formation of the fat-ring. Apparently, pasteurization temperatures ranging from 150° to 190°F. have no decided effect on the tendency for homogenized milk to form a fat-ring. As was indicated by the data in Table 1, data in Table 3 also show that the temperature at which the milk was homogenized, especially with respect to the temperature of pasteurization, was an important factor in the formation of the fat-ring. Photographs

TABLE 2

The composition of fat-ring material when milk pasteurized at 190°F. for 30 minutes was homogenized at 3000 + 500 pounds pressure at various temperatures

Homogenization temperature	Analysis of fat ring material			Prominence of fat-ring after one week at 40°F.
	Fat	Solids-not-fat	Ash	
(°F.)	(%)	(g./100 g. fat)	(g./100 g. fat)	(degree)
Homogenized after pasteurization				
190	No fat-ring formation			0
170	No fat-ring formation			0
150	55.34	8.62	.832	1
130	43.32	10.76	1.026	2
110	61.94	7.10	.520	2
90	65.94	13.03	.437	4
70	58.67	11.86	.336	4
Homogenized after 24 hr. storage at 40°F.				
70	60.11	7.12	.436	4
90	62.25	9.63	.426	4
110	63.30	9.80	.419	4
130	64.32	5.77	.443	3
150	(68.64% total solids)[1]			1
170	55.36	9.46	.613	1

[1] Sample for fat test lost.

Legend: no fat ring = 0
slight fat ring = 1
distinct fat ring = 2
prominent fat ring = 3
cream plug = 4

TABLE 3

Degree of fat-ring formation on milk pasteurized and homogenized at different temperatures

Pasteurization temperature (30 min.)	Homogenization temperature (3000 + 500 lbs.)	Trial				
		A	B	C	D	Average
(°F.)	(°F.)	(degree)	(degree)	(degree)	(degree)	(degree)
150	150	2	2	2	2	2.00
	130	3	3	3	2	2.75
	110	4	4	4	4	4.00
170	170	1	1	2	1	1.25
	150	3	2	2	1	2.00
	130	3	3	3	3	3.00
	110	4	4	4	4	4.00
190	190	1	1	1	2	1.25
	170	1	1	1	2	1.25
	150	2	2	2	2	2.00
	130	3	3	4	4	3.50
	110	4	4	4	4	4.00

Legend: no fat ring = 0
slight fat ring = 1
distinct fat ring = 2
prominent fat ring = 3
cream plug = 4

of the typical fat-ring formation on milk homogenized at various temperatures are shown in Figure 3.

Composition. Pooled milk pasteurized by the holding process was obtained prior to cooling from the College Creamery, brought to the laboratory, and divided into 4 portions. The first portion, serving as a control, was homogenized. To the other 3

TABLE 4

The influence of increasing fat concentrations upon degree of fat rising on homogenized milk after seven days storage at 40°F.

Analysis of original milk		Degree of fat rising on milk with the addition of increasing increments of table cream[1] (*lbs./gal.*)				
Fat	Solids-not-fat	0.0	0.5	1.0	1.5	2.0
(%)	(%)					
3.20	7.71	3	4	4	4	4
3.27	7.87	3	3	3	3	4
3.17	7.60	0	3	3	3	4
3.75	8.43	0	2	2	3	3
3.63	8.02	2	3	4	4	4

[1] Test of cream ranged from 19.6 to 18.6% fat.
Legend: no fat ring = 0
slight fat ring = 1
distinct fat ring = 2
prominent fat ring = 3
cream plug = 4

TABLE 5

The influence of increasing solids-not-fat concentrations upon the degree of fat rising on homogenized milk after seven days storage at 40°F.

Analysis of original milk		Relative degree of fat-ring formation with the addition of increasing increments of SNF-water mixtures[1] (*lbs./gal.*)				
Fat	Solids-not-fat	0.0	0.5	1.0	1.5	2.0
(%)	(%)					
3.32	7.85	1	1	2	1	2
3.69	8.76	4	4	4	4	4
4.00	9.02	4	4	3	4	4
3.39	7.73	0	1	1	2	3
3.65	8.75	1	3	3	3	4
3.75	8.74	3	4	3	3	(lost)
Average estimate of fat-ring formation		2.16	2.83	2.66	2.83	3.40

[1] SNF—water mixture contained 25% solids-not-fat.
Legend: no fat ring = 0
slight fat ring = 1
distinct fat ring = 2
prominent fat ring = 3
cream plug = 4

portions various quantities of cream and/or nonfat dry milk solids were added prior to homogenization. All samples were homogenized at 2000 + 500 p.s.i. in a 75 gal./hr., Manton-Gaulin, 2-stage homogenizer at 135°F. Data presented in Tables 4 and 5 show that the addition of cream or solids-not-fat following pasteurization and prior to homogenization results in an increased tendency for homogenized milk to show a fat-ring formation. Data in Table 1 also seem to emphasize the importance of the fat concentration in the formation of a fat-ring. The higher the fat concentration of milk the greater was the tendency of the milk to exhibit a fat-ring.

DISCUSSION AND SUMMARY

The factors relating to the formation of a fat-ring defect on homogenized milk which were studied in this report indicate that the fat-ring results from the accumulation of nonhomogenized fat globules and/or poorly emulsified fat (free-fat) *(1)*. This conjecture is supported by the analysis of

the fat-ring material reported in Table 2. The apparent general decrease in the concentration of solids-not-fat and ash per unit of constituent fat indicates that less casein (if we assume the source of ash in this case to be casein) and/or other protein materials are associated with the fat-ring material gathered from milk showing the greatest fat-ring formation. Further, when fat-ring material was heated, a considerable amount of melting-off was observed, suggesting the presence of poorly stabilized fat.

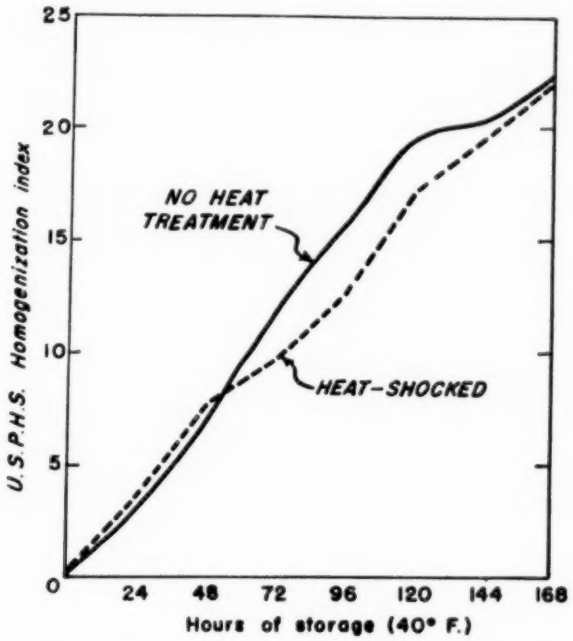

Figure 2. United States Public Health Service Homogenization index on milk of different ages, some of which was warmed to room temperature 24 hours before sampling.

Homogenization at higher-than-normal pressures and temperatures tends to lessen the fat-ring formation. While high homogenization pressures act as a stabilizing influence on the milk fat, one would not expect that higher-than-normal homogenization temperatures would have a similar effect unless certain of the heat-labile serum proteins were more readily immobilized on the fat-globule surface at these higher-than-normal temperatures. Possibly the ability of the milk fat to adsorb stabilizing proteins from the plasma is increased with increasing temperatures. At relatively low temperatures of homogenization, the development of the fat-ring might be accounted for on the basis of fat agglomeration, since temperatures in the range of 80°F. to 110°F. are conducive to fat clumping and agglomeration *(1, 2)*.

Doan *(3)* stated that so long as the fat/solids-not-fat ratio was sufficiently low, below 0.6 to 0.85 for cream, there would be a reduction in the

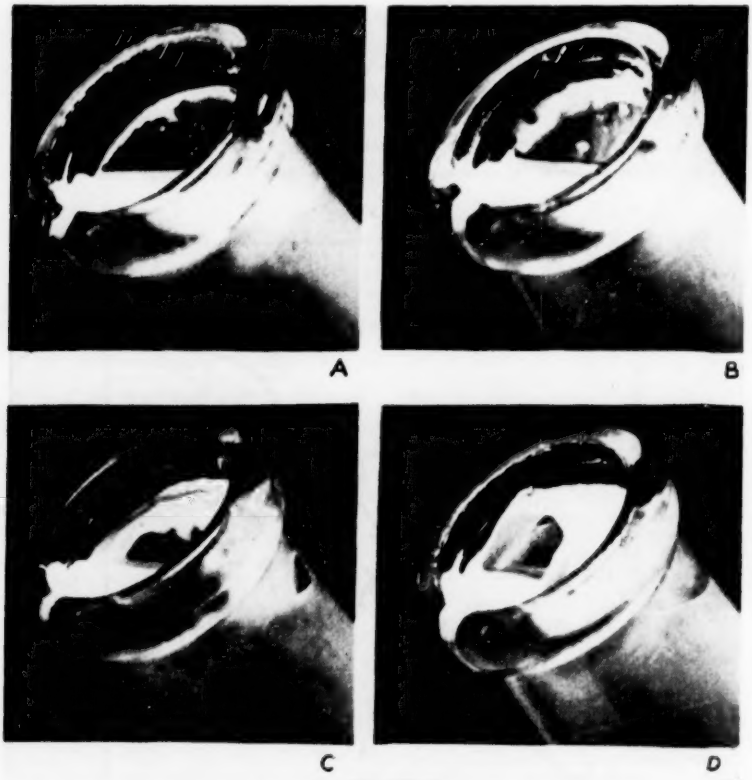

Figure 3. **The influence of homogenization temperature on the formation of a fat-ring on milk pasteurized at 170°F. when homogenized at 170°F. (A), 150°F. (B), 130°F. (C) and 110°F. (D).**

tendency for fat globules to clump in homogenized cream. Because of these observations it was not expected that an increased fat-ring formation would result on homogenized milk to which additional nonfat dry milk solids had been added. Possibly, the increase in the specific gravity of the plasma, resulting from the dispersion of additional nonfat dry milk solids accentuated the rise and accumulations of the poorly homogenized fat particles on homogenized milk.

Observations made throughout this study have indicated that the concentration of fat in the homogenized milk was a definite factor in the formation of a fat-ring, the higher fat milks showing the greatest susceptibility to the defect. The importance of the concentration of milk fat to the relative viscosity and a "chalky" flavor defect in homogenized milk which was thought to be associated with the profuse clumping of fat globules was reported previously *(1, 5)*.

CONCLUSIONS

A defect of homogenized milk known as "fat-ring" has been described. The material was found to be composed largely of milk fat which was present in a highly de-emulsified state.

Heat-shocking accelerated the rate of rise of fat in homogenized milk provided the treatment was administered within 72 hours following bottling. However, this treatment did not enhance materially the formation of a fat-ring.

Homogenization of milk at temperatures markedly lower (110° F.) than those of pasteurization were conducive to fat-ring formation. Higher-than-normal homogenization temperatures and pressures were a deterrent to its formation.

The addition of nonfat dry milk solids, as well as milk fat (19% cream), to milk prior to homogenization seemed to stimulate the formation of a fat-ring on homogenized milk.

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RELATION OF ORGANIC NITROGEN CONTENT OF THE MUST TO COLLOIDAL STABILITY OF THE YOUNG WINE[a]

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(Manuscript received June 28, 1954)

In winery operation it was often noticed that young white wines varied greatly in their rate and degree of natural clarification, and that certain wines would require much larger amounts of fining agents than others to achieve the same degree of clarity and colloidal stability. An attempt was therefore made to determine if any specific chemical component of the grape must used in making these wines was an influencing factor on their later clarification and stability. If so, did that component of the must correlate in any maner with *(a)* variety, *(b)* degree of maturity, *(c)* age of vineyard, *(d)* location of vineyard or any other influencing factor. Also what could be done to these musts, if anything, to achieve easier and better colloidal stability in the young wines.

EXPERIMENTAL

In the 1947 vintage a number of white musts were analyzed for degree Balling, total acid, pH, tannin and coloring matter, iron, copper and total organic nitrogen. All analyses except that for nitrogen were determined as prescribed in the University of California *Laboratory Procedures for Enology*. The organic nitrogen analysis was carried out according to the micro-kjeldahl method outlined in *Methods of Analysis*, A.O.A.C.

This group of musts included the following varieties: Semillon, White Riesling, Traminer, Sylvaner, Sauvignon vert, White Pinot, Pinot Chardonnay and Melon de Bourgogne. There were 12 vineyards represented with each sample of a given variety coming from different locations.

After the wines had finished their normal alcoholic fermentation, and all had passed through normal cellar procedure they were examined for clarity and colloidal stability. This examination occurred following the second racking—approximately 90 days after the must analysis. As removed from the tanks, all wines were relatively clear, with no conspicuous differences in clarity. Samples of these wines were then subjected to a temperature of 140°F. for 24 hours to coagulate the residual protein material, and then held at room temperature for 24 hours before being evaluated for stability.

Those wines which remained clear with no evidence of sediment were given an arbitrary rating of No. 1. Those wines which developed a specific haze or light cloudiness were classified as No. 2. Wines with a heavy haze (and which threw a deposit upon further standing) were called No. 3, and the wines that were completely hazy with a heavy coagulant material in suspension and a cheesy sediment were graded No. 4.

RESULTS AND DISCUSSION

Of all the must constituents analyzed, nitrogen content was the only one which gave a significant correlation with stability. The correlation coefficient obtained was +0.6598, corresponding to a probability value of

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[a] Presented at the Fourteenth Annual Meeting of IFT, Los Angeles, California, June 28, 1954.

[b] Present address: United States Army.

TABLE 1

Relationship of nitrogen content of the must to stability of the wine

Variety	Vineyard	Nitrogen mg./liter	Stability
Semillon	4	70	1
	2	101	4
	5	157	4
	6	252	4
	3	315	4
Sauvignon vert	4	42	1
	5	42	1
	8	49	1
	1	54	1
	7	154	2
White Pinot	10	28	1
	8	42	1
	11	63	2
	9	119	3
Franken Riesling	5	35	1
	12	63	1
	10	77	1
	2	145	3
White Riesling	10	84	1
	2	91	1
Pinot Chardonnay	10	40	1
	3	274	4
	1	315	4
Traminer	10	28	1
	8	108	3
Melon de Burgogne	3	154	3

< 0.001 which shows the existence of a highly significant relationship. As can be seen in Table 1, increasing nitrogen content of the must in general results in wines of decreasing stability.

Nitrogen content of the must appears to be dependent to some extent on grape variety and location where grown. The nitrogen content of the Sauvignon vert and White Pinot is considerably lower than that of the Pinot Chardonnay and Semillon. It is also noted that grapes from vineyards 4 and 10 (Table 1) are consistently lower in nitrogen than those from other locations. It is thought that this quite possibly could be due to variations in soil types, as no effect of climatic differences or vine age could be demonstrated.

From the limited data available it is impossible to fix the maximum amount of nitrogen which would allow easy clarification and assure a stable wine. However, from inspection of the data in Table 1 it appears that musts containing less than 100 mg. of nitrogen per liter would usually produce stable wines.

Table 2 gives the results of pectic enzyme treatment of several musts. The added enzyme substantially reduced the nitrogen content, and increased

TABLE 2

Effect of Pectinol on nitrogen content of must and on stability of the wine

Variety	Vineyard	Pectinol lbs./1000 gals.	Nitrogen mg./liter	Stability
Semillon	2	0	101	4
		2	84	3
		3	40	2
Semillon	3	0	315	4
		3	54	2
Melon de Burgogne	3	0	154	3
		2	126	2
White Pinot	9	0	119	3
		2	63	2

the stability of the resultant wines. However, the use of pectic enzymes cannot be unqualifiedly recommended in all wines as their use has occasionally resulted in increased browning.

SUMMARY

A high degree of correlation was found between nitrogen content of must and the instability of the resultant wines. The nitrogen content appears to be at least partially dependent upon variety and vineyard location. The addition of pectic enzymes to the must results in lowered nitrogen content and increased stability.

STERILIZATION OF CUCUMBERS FOR STUDIES ON MICROBIAL SPOILAGE[a, b]

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(Manuscript received July 1, 1954)

Final proof of the etiological relationship between bacteria, molds, and yeasts and the spoilage of cucumber pickles must involve pure culture studies with sterile cucumbers whose original physical condition has not been significantly altered. In the past, few attempts have been made to apply Koch's postulates in their entirety to spoilage problems involving fresh, fermenting, or fermented cucumbers. In these cases, sterilization was attempted by the use of heat.

Heating may have an adverse effect on the firmness of pickles *(4, 6)*. This fact has been recognized in development of pasteurization procedures for preservation of various types of commercially packed pickles *(2, 8)*. These acid products, pasteurized at temperatures between 71.1°C. (160°F.) and 85°C. (185°F.) for about 15 to 45 minutes, contain comparatively few microorganisms[c] but are not sterile *(1, 3, 5)*.

Because the usual methods of sterilization by autoclaving (121°C.; 249.8°F.) or intermittent steaming (100°C.; 212°F.) caused severe chemical and physical changes in cucumbers, an attempt was made to use lower temperatures for sterilization. Intermittent heating at 71.1°C. (160°F.) for 30 minutes on 3 or more successive days did not markedly reduce the original firmness of the cucumbers. However, sterilization was not always obtained. Therefore, a more reliable method was sought.

EXPERIMENTAL METHODS AND RESULTS

Effect of heat on the physical structure of cucumbers. Since sterilization was seldom obtained by intermittent heating at 71.1°C. (160°F.) the possibility of using somewhat higher temperatures was investigated. First, however, it was necessary to determine what effect the contemplated increase in temperature would have on the physical structure of cucumbers.

For this purpose salt stock cucumbers (sizes 2 and 3) were immersed in a water bath set at the desired temperature. Samples were removed at different intervals and plunged into ice water. Then the firmness was determined with the pressure tester as described by Jones and Etchells *(7)* with the exception that the middle portion of each cucumber was tested as well as the blossom and stem ends. Another portion of each sample was investigated for other defects which might result from the heating. Temperatures of 71.1°C., 82.2°C., and 93.3°C. (160°F., 180°F., and 200°F.) were used. In one series the cucumbers were immersed in tap water adjusted to pH 6.4 to 6.6. In a second series the original brine (10% salt; 1.0% acid as grams lactic acid per 100 ml. of brine; and pH 3.5) was used as the heating medium. Each series was investigated at

[a] Support of this study from a grant-in-aid to the University of California by the American Spice Trade Association is gratefully acknowledged.

[b] Presented at the Fourteenth Annual Meeting of IFT at Los Angeles, California, July 1, 1954.

[c] These surviving organisms do not spoil the pasteurized products because they cannot grow in an acid environment.

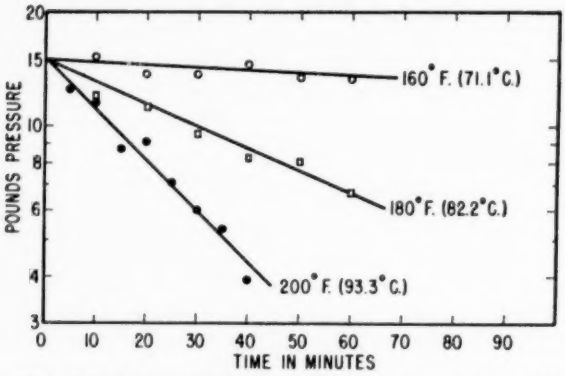

Figure 1. The effect of temperature on reduction in firmness of cucumbers as measured by the pressure tester (7).

least twice at each temperature. The rate of heat penetration to the center of the cucumbers was determined by thermocouple measurements.

Significant softening of the cucumbers occurred at temperatures above 71.1°C. (160°F.). The rate of softening was essentially the same regardless of the heating medium—water or brine. As shown in Figure 1 the loss in pressure of the cucumbers was accelerated as the temperature of heating was increased.

Heating also caused the formation of gas pockets in cucumbers as shown in Figure 2. These pockets were not detected in cucumbers heated at 71.1°C. (160°F.) but were found in all cucumbers examined that had been heated at 82.2°C. (180°F.) and above. Subsequently, it was discovered that similar pockets could be formed at will by placing cucumbers in a vacuum. Therefore, it may be that this defect is caused by the rapid expansion of dissolved gases present in the pickles. In any event, it is obvious that intermittent heating at a temperature high enough to sterilize the cucumbers will result in simultaneous softening and pocket formation.

The use of chemical sterilizing agents. Attempts were made to sterilize the surface of the cucumbers by chemicals including mercuric chloride, sodium hypochlorite, ethanol, and propylene oxide, respectively. Desalted cucumbers were immersed in aqueous solu-

Figure 2. Gas-pocket formation in cucumbers.
Left, heated at 82.2°C. (180°F.) for 15 to 20 minutes.
Center, control cucumber.
Right, under vacuum for 3 to 5 minutes.

tions containing various concentrations of these agents for different lengths of time, rinsed several times in sterile water, and then transferred to sterile, buffered, 6% salt solution contained in 500 ml. wide-mouth, cotton-plugged bottles. The criterion for sterility used was absence of growth in the brines with higher pH values (5.5 to 7.0) after incubation at 35°C. (95°F.) for 3 to 4 weeks. Sterility was never obtained.

Additional experiments were performed with various concentrations of propylene oxide added directly to buffered brine with submerged cucumbers. Under these conditions the propylene oxide caused a considerable increase in the pH value of the brine, probably because of the rapid hydrolysis of the epoxide in water. This treatment also caused undesirable softening of the cucumbers. Too, in many cases, sterilization was not achieved.

An attempt also was made to use the combined effect of mild heat and low pH values to obtain sterile cucumbers. For these tests desalted cucumbers were added to sterile brine (pH 3.2 to 3.8), and heated at 71.1°C. (160°F.) for 30 minutes on 3 successive days. Then sterile sodium hydroxide was used to adjust the pH of the cucumbers and brine to the desired value in the range of 5.0 to 6.5. Growth was not observed until after the pH adjustment was made. Then, within 2 days, the surviving organisms produced marked turbidity in the brines.

The use of antibiotics. Antibiotics were investigated because the other methods for sterilization of cucumbers in brine had failed. Exploratory experiments with a number of these products gave promising results. Penicillin, the first antibiotic selected for detailed investigation, was chosen because its residual could be readily neutralized. However, it soon became apparent that penicillin used alone was not completely effective against film forming yeasts. Thereafter, it was used in combination with mild heat.

Sterilization of the cucumbers was fulfilled by first autoclaving 250 ml. of brine in 500 ml. cotton-plugged, wide-mouth bottles. The brine contained 6.0% sodium chloride which had been added to 0.1 *M* lactate-phosphate buffer at the desired pH. In some experiments 25 ml. of fresh cucumber homogenate was added to the brine. After cooling, penicillin[d] was added in a concentration of 2000 units per ml. of brine, three fresh or desalted cucumbers were submerged in the brine, and the bottles and contents were incubated at 35°C. (95°F.) for 4 to 8 hours. Then the treated cucumbers were heated at a brine temperature of 71.1°C. (160°F.) for 30 minutes and incubated for 3 or more days before sterile penicillinase[e] was added. After additional incubation to allow for sterility tests, the cucumbers, if sterile, were ready for inoculation with pure cultures.

Additional experience showed that penicillin resistant, pectinolytic, aerobic, spore-forming bacteria sometimes survived. This difficulty was overcome by the combined effects of penicillin and streptomycin[f] used in concentrations of 1000 units per ml. and 200 μg. per ml., respectively, in conjunction with mild heat (71.1°C.; 160°F.). The remainder of the revised sterilization procedure was the same as that previously described.

This method has some serious disadvantages. The interference of the residual streptomycin must be overcome by training the desired test cultures to grow in its presence.[g] (However, species of *Bacillus*, the only microorganisms studied until now, have been trained without difficulty to tolerate at least 500 μg./ml. of the compound.) Furthermore, it is possible that contaminating heat-resistant microorganisms may become adapted to both antibiotics or the antibiotics may cause a change in the characteristics of the trained test cultures. The possibility also exists that some test organisms cannot be trained to tolerate streptomycin. However, the variable results obtained with other treatments make it mandatory that this or a similar method be used for sterilizing cucumbers to be used for pure culture studies where Koch's postulates must be applied.[h]

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[d] Penicillin G sodium, Merck and Co.

[e] Nutritional Biochemicals Corp.

[f] Calcium chloride complex, Merck and Co.

[g] Streptomycin can be inactivated by cysteine and hydroxylamine.

[h] It is not clear at the present whether radiation can supplant this or a similar method for sterilization of cucumbers in brine. However, since this manuscript was prepared it has been found that 50μg. per ml. of aureomycin used in combination with mild heat as described above will sterilize cucumbers.

SUMMARY

It is necessary to use pure cultures and sterile cucumbers whose original physical structure has not been significantly altered when attempting to determine the etiological relationship of microorganisms to softening or other spoilage. The usual methods of sterilization by autoclaving or intermittent steaming cause severe softening and gas-pocket formation in the cucumbers. A method involving the use of mild heat (71.1°C.; 160°F.) in combination with penicillin (1000 units/ml. of brine) and streptomycin (200 μg./ml. of brine) is described which may be used to sterilize cucumbers without significantly altering their texture.

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NUTRIENT COMPOSITION OF FRESH AND FROZEN LIMA BEANS IN RELATION TO VARIETY AND MATURITY

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The production of lima beans for processing has increased steadily during the last few years in the United States as a whole and especially in California. The 1953 crop in California was 40,300 tons and in the United States 105,900 tons, representing monetary values of $4,484,000 and $16,206,000, respectively *(19)*. Because of the rapid increase in consumption of frozen lima beans, it is desirable to have more complete information on nutrient composition and organoleptic quality as influenced by variety, species, maturity, locality of growth, and the freezing process.

Among so-called baby lima beans, Bailey *(2)* has described the climbing type of baby (sieva) limas as *Phaseolus lunatus*, L. and the bush type as *Phaseolus lunatus*, L. var. *lunonanus*, Bailey. "Wilbur" is a climbing type of baby lima; "Cangreen" and "Clark's Bush" are of dwarf or bush form (Hendersons). Cangreen and Clark's Bush are similar in appearance and possess the green cotyledon character. These beans do not become white during maturation but are greenish at the drying stage. "U. S. 242" and "Concentrated Fordhook" (potato lima) are described as *Phaseolus limensis*, Macf. var. *limenanus*, Bailey. Several years earlier Mackie *(11)* designated all the above types of lima beans as *Phaseolus lunatus*. In many studies of the composition of lima beans, the variety and the species were not identified.

Of the numerous investigations of the nutritive value of fresh and frozen lima beans, the majority have reported on one or just a few of the constituents. Only a few papers have dealt with the vitamin, mineral, and proximate compositions. Munsell *et al. (13)* analyzed 13 constituents of lima beans *(P. limensis)* grown in Honduras. The values cited by Watt and Merrill *(18)* are compiled from various sources and do not represent any one sample.

Since a thorough survey of the nutrient components is not available, a reinvestigation of lima bean composition seemed desirable.

EXPERIMENTAL PROCEDURE

Materials. Cangreen and U. S. 242 varieties were grown at Davis during the summer 1952. The beans were harvested in the morning, separated from the pods with a mechanical podder, and prepared for analysis on the same day. Three harvests were made to obtain the physiological maturities described below. The first harvest yielded Maturity Classes I and II; the second yielded Maturity Class III; and the third yielded the dry beans.

Clark's Bush and U. S. 242 were selected from commercially grown fields in Patterson, Stanislaus County. The plants were harvested during the early hours of the morning

(2-3 A.M.) so that the beans could be prepared for analysis on the same day at Davis. After commercial vining in the field, the beans were taken to the processing plant. All three maturity classes were collected from the processing line after the beans had gone through a screening process in which broken beans, pods, dirt, and other debris were removed. The samples were transported to Davis in a refrigerated box. The time lapse between harvest and arrival at the laboratory was approximately 5 to 6 hours.

TABLE 1

Physiological maturity class	Baby type		Fordhook	
	Sieve size	Approx. wt. per bean	Sieve size	Approx. wt. per bean
I Immature	less than 7/16"	0.30 gm.	less than 5/8"	0.95 gm.
II Optimum for freezing (green color)	greater than 7/16"	0.70 gm.	greater than 5/8"	2.00 gm.
III Slightly more mature than II (pale green color)	greater than 7/16"	0.80 gm.	greater than 5/8"	2.50 gm.

The beans were passed through sieves for sorting into 3 physiological maturities as described in Table 1. Maturity Class I includes the smaller, immature beans that had not reached maximum size; Class II beans constitute the bulk of the U. S. Grade A package. Class III contains more mature, well-filled beans which are still green and would pass the color standard for U. S. Grade A frozen beans.

The Maturity Class III beans were selected by visual inspection after the screening process. In the Cangreen variety from Davis, pod appearance was used as an aid in the selection for this maturity. The pods generally had begun to lose a small amount of the green color and had become slightly dry. Further selections were made after pod removal to eliminate the younger and the partially dried beans.

No difficulty was encountered in selecting the Maturity Class III beans in the Fordhook varieties since these beans were well filled and were turning yellowish-green or losing some of their green color. No dry or white beans nor any with wrinkled seed coats were selected in Maturity Class III.

Commercially packaged U. S. Grade A beans contain different percentages of these maturities; the percentages vary according to the physiological maturity of the field.

The graded beans were immediately separated into two lots, one of which was analyzed fresh and the other frozen for organoleptic tests and for nutrient value studies. After being steam blanched at 212°F. (100°C.) for 3 minutes and cooled in a tap water spray, the beans of the second lot were sealed into cans and frozen at -20°F. (-29°C.). Some were packed into No. 2½ cans for food value studies, others into 8-oz. cans for taste tests. These cans were stored at -20°F. (-29°C.) for 3 to 4 months and analyzed.

In addition, commercially frozen beans were obtained from the major lima bean-producing areas of California. Among these were Clark's Bush and U. S. 242 beans grown at Patterson, which came from the same lots as the samples described above. According to our classification of maturity, the Clark's Bush from Patterson had the following approximate composition: I = 13%, II = 64%, and III = 23%, and the U. S. 242 the following: I = 28%, II = 59%, and III = 13%. These two samples were U. S. Grade A and had respective color scores of 58 and 54. Grade A frozen lima beans must have a color score between 54 and 60 *(20)*.

Organoleptic tests. A balanced incomplete block design described by Cochran and Cox *(5)* was used in the organoleptic analysis of the frozen samples. The design involves judging only part of the total number of samples at one time, each sample being compared with every other sample an equal number of times. There were 21 judging periods, 5 samples judged each period, and 5 replications of each sample, with two samples appearing together once in the same block. Blocks and serving order were randomized throughout the experiment. The samples were scored on a scale of 7 points: score 7—*smooth, moist*; score 1—*dry, granular (15)*. Six trained judges participated in the scoring.

The frozen beans were weighed in beakers, and boiling salted water (0.5% NaCl) was added in the proportion of 150 ml./90 g. All samples were cooked 12 minutes after

boiling had started. The water was drained immediately after cooking, and the 5 samples were served as soon as possible.

Analytical methods. Approximately 1000 g. of fresh or frozen lima beans were chopped with stainless steel knives, and duplicate samples were taken for all constituents to be analyzed. Vitamin analyses were made immediately. Samples were preserved in boiling 95% alcohol for carbohydrate and alcohol insoluble solids determinations. Dry samples were prepared for total solids, protein, fat, and mineral analyses.

Total solids: Fifty g. of the chopped sample were dried in a forced-draft oven at 65° to 70°C. for 36 to 48 hours and reweighed. This weight was taken as total solids.

Carbohydrates: Total sugars and starches were determined by the official methods *(14)*. Starches were determined on the 80% alcohol extract residue using diastase instead of malt extract for digestion.

Alcohol-insoluble solids: Residue of the Soxhlet extractions with 80% alcohol for sugar determinations dried in a forced draft oven at 70°C. was taken for A.I.S.

Crude fat: Determined by extracting 2 g. of dry ground sample with ethyl ether. The loss in weight was taken as fat.

Protein, calcium, phosphorus, and iron: These were determined by the official methods *(14)*. Amino-naphthol sulfonic acid was substituted for hydroquinone as the reducing agent in the determination of phosphorus.

Vitamin A: Vitamin A value was calculated from carotene determinations, where 0.6 microgram of beta carotene or 1.2 micrograms of alpha carotene equal one I.U. Carotene was determined by the method given in *Methods of Vitamin Assay (1)*. The sample was extracted in alcoholic KOH and petroleum ether in a Waring blender. Chromatographic analysis was used.

Ascorbic acid: Ascorbic acid was determined by the method of Loeffler and Ponting *(12)*. Dehydro-ascorbic acid, which was reduced to ascorbic acid by H_2S, was included.

Thiamine and riboflavin: Thiamine and riboflavin were determined photofluorometrically *(1)*.

Niacin: A microbiological method *(14)* was used. Enzyme-hydrolyzed casein was used in the basal medium.

RESULTS AND DISCUSSION

The nutrient composition and organoleptic scores for beans of selected maturities are summarized in Tables 2 and 3. Included in Table 2 are the calculated average amounts of nutrients in the 12 vegetables with the highest per capita consumption, adjusted for per capita consumption of each vegetable *(21)*.

The immature beans (Maturity Class I) scored higher in the organoleptic tests than those of Maturity Classes II and III. In the fresh bean samples the total solids, alcohol-insoluble solids (A.I.S.), starch, and proteins increased with maturity, whereas the total sugar content decreased. Ascorbic acid and carotenes decreased with maturity, while little change occurred in the B vitamins, thiamine, riboflavin, and niacin.

Blanching and freezing caused little change in total solids, A.I.S., starch, proteins, and fats. These results agree with those of Kramer and Smith *(8)* who found no appreciable change in the proximate analyses as the result of steam blanching. The total sugar content, however, decreased on blanching and freezing. The phosphorus content of the blanched and frozen beans was lower than that of the fresh samples. Lower sugar and phosphorus values might be due to leaching when the blanched samples were cooled with tap water before being sealed into cans and frozen.

In some cases there were large differences in vitamin content between

TABLE 2

Baby type lima beans — per 100 g. fresh edible portion

Sample	Maturity	Organoleptic score[1]	Total solids	Alcohol insoluble solids	Total sugars	Starch	Protein	Fat	Ca	Fe	P	Carotene alpha	Carotene beta	Vit. A I.U.	Ascorbic acid	Thiamine	Riboflavin	Niacin
		mean	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*
Cangreen Variety—Grown at Davis, California																		
Fresh	I		22.6	16.1	3.5	5.3	5.9	1.0	59	2.8	141	0.13	0.33	660	38	0.30	0.16	1.7
Frozen	I	5.8	22.1	17.0	2.7	5.6	5.5	1.1	52	2.8	113	0.12	0.31	620	19	0.16	0.12	1.3
Fresh	II		34.4	28.9	2.6	7.4	7.6	1.5	60	3.5	173	0.05	0.17	320	28	0.27	0.12	1.6
Frozen	II	3.4	33.3	29.1	1.4	9.8	7.6	1.9	55	3.6	156	0.04	0.10	200	16	0.15	0.09	1.4
Fresh	III		41.7	37.7	2.8	12.9	8.3	1.8	65	3.7	208	0.01	0.14	240	23	0.24	0.10	1.4
Frozen	III	3.0	39.8	34.5	1.4	10.8	8.1	1.8	66	3.5	177	0.02	0.13	230	10	0.15	0.08	1.2
Fresh	Dry beans	....	91.6	80.3	4.4	22.5	18.1	4.3	139	7.7	454	0.00	trace	trace	0	0.56	0.14	1.5
Clark's Bush Variety—Grown at Patterson, California																		
Fresh	I		23.6	19.4	2.8	6.4	5.7	0.9	71	3.2	115	0.09	0.24	480	21	0.22	0.14	1.4
Frozen	I	4.2	23.4	20.8	2.0	6.7	5.8	0.9	71	3.4	96	0.11	0.26	520	20	0.15	0.11	1.1
Fresh	II		36.6	34.1	1.2	10.5	8.6	1.5	69	2.8	145	0.06	0.14	280	11	0.11	0.09	1.2
Frozen	II	2.7	35.3	32.2	0.8	9.1	8.4	1.5	83	2.8	130	0.02	0.09	170	13	0.10	0.08	0.9
Fresh	III		40.8	37.9	1.3	11.3	9.5	1.6	38	3.1	166	0.02	0.07	130	9	0.12	0.09	1.2
Frozen	III	2.8	39.1	36.4	0.8	10.0	9.3	1.8	58	3.4	149	0.01	0.06	110	10	0.12	0.08	0.9
Average of 12 vegetables with highest per capita consumption.[2]			13.1	60	Kg. cal. energy		1.6		24	0.9	44			630	20	0.08	0.07	0.8

[1] Measurement of texture on scale of 7 points: Score 7—*smooth, moist;* Score 1—*dry, granular.*

[2] The per capita consumption of these vegetables is potato 110.0 lbs., cabbage 33.4; tomato 23.9; watermelon 23.5; onion 21.3; lettuce 17.3; sweet potato 13.8; sweet corn 12.5; carrot 11.7; celery 10.2; cantaloupe 9.2; and snap bean 9.0. The average is adjusted for per capita consumption of different vegetables.

TABLE 3

Fordhook type lima beans — per 100 g. edible portion

Sample	Maturity	Organoleptic score[1]	Total solids	Alcohol insoluble solids	Total sugars	Starch	Protein	Fat	Ca	Fe	P	Carotene alpha	Carotene beta	Vit. A I.U.	Ascorbic acid	Thiamine	Riboflavin	Niacin
		mean	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*
U.S. 242 Variety—Grown at Davis, California																		
Fresh	I		19.4	15.5	3.1	5.0	4.9	1.8	34	1.8	119	0.16	0.25	550	36	0.27	0.13	1.4
Frozen	I	6.8	21.2	17.1	2.2	6.3	4.7	1.2	37	2.0	100	0.11	0.23	470	23	0.18	0.10	1.3
Fresh	II		30.1	26.0	2.7	7.7	6.3	1.9	37	2.4	126	0.05	0.13	260	27	0.24	0.10	1.6
Frozen	II	5.4	28.9	23.0	2.3	8.7	5.6	1.7	38	2.4	107	0.03	0.10	190	15	0.17	0.08	1.4
Fresh	III		38.9	34.3	1.8	10.4	7.6	1.6	40	3.1	165	0.00	0.05	80	18	0.33	0.09	1.1
Frozen	III	4.8	35.4	32.0	0.8	10.4	6.9	1.4	53	3.0	148	0.00	trace	trace	7	0.22	0.06	0.9
Fresh	Dry Beans		84.6	77.9	2.8	32.0	15.9	4.4	90	9.8	377	0.00	0.00	0	0	0.62	0.14	1.5
U.S. 242 Variety—Grown at Patterson, California																		
Fresh	I		21.0	17.3	2.5	5.8	5.1	1.5	35	2.1	94	0.10	0.17	370	27	0.26	0.11	1.4
Frozen	I	5.6	21.8	18.0	2.2	7.0	5.1	1.1	41	2.2	81	0.09	0.17	360	20	0.15	0.08	0.8
Fresh	II		29.3	27.4	2.0	9.0	6.6	1.8	44	2.2	120	0.01	0.14	240	23	0.22	0.09	1.5
Frozen	II	5.1	28.9	24.8	1.5	8.3	6.6	1.4	42	2.3	101	0.01	0.09	160	18	0.15	0.07	0.7
Fresh	III		34.6	32.4	1.6	9.3	8.0	2.1	42	2.8	128	0.00	0.07	120	18	0.24	0.08	1.3
Frozen	III	4.0	33.0	29.4	1.2	9.0	7.6	1.5	37	2.9	113	0.00	0.05	80	17	0.17	0.07	0.8

[1] Measurement of texture on scale of 7 points: Score 7—smooth, moist; Score 1—dry, granular.

the fresh samples and the frozen samples. The ascorbic acid loss was occasionally as high as 50%. Although blanching and freezing usually caused a decrease in the ascorbic acid content, the samples of Clark's Bush from Patterson showed no change. This apparent high retention of vitamin C in the frozen beans could be due to losses in the fresh material during transportation to Davis inasmuch as similar samples from Davis which did show such loss in blanching had higher initial ascorbic acid content. These results are in agreement with those of Caldwell *et al. (4)* and also Kramer and Mahoney *(7)* who reported large losses of vitamin C due to blanching in several varieties of lima beans. The content of carotene, thiamine, riboflavin, and niacin was lower after blanching and freezing in all maturities tested. The data of Wagner *et al. (16, 17)*, when recalculated on the fresh basis, showed similar losses of vitamins as the result of blanching. Guerrant *et al. (6)*, working with all-green baby limas (hybrid variety—Fish's special), observed little loss of ascorbic acid and riboflavin but found that the thiamine content dropped from 0.22 mg. to .17 mg. per 100 g. of sample and the niacin content from 1.9 mg. to 1.2 mg. per 100 g. during 5 minutes of steam blanching.

The carotene content of green mature lima beans is quite low, as pointed out by Zscheile *et al. (23)*. The total carotene content dropped from 0.46 mg. in immature beans to just a trace in the dry beans of the Cangreen variety. This same trend was observed from immature to dry beans for the other variety tested. In agreement with the results of Zimmerman *et al. (22)*, two carotenoid pigments, about one-third alpha and two-thirds beta carotene, were found. No determination of neo-beta carotene *(3)* was made.

Because total solids, A.I.S., starch, protein, and Vitamin C (ascorbic acid) contents have been suggested as indices for establishing the maturity and quality of lima beans, coefficients of correlation were calculated from the results of Tables 2 and 3. These coefficients are presented in Table 4. It would appear from these results that physiological maturity as expressed

TABLE 4

Coefficients of correlation of values from Tables 2 and 3

Constituent	Organoleptic score[1]	Alcohol-insoluble solids[2]
Baby Lima Bean		
A.I.S.	−.94	....
Total solids	−.90	+.99
Starch	−.90	+.98
Protein	−.91	+.99
Vitamin A value	+.94	−.79
Ascorbic acid	+.80	−.72
Fordhook Type		
A.I.S.	−.85	....
Total solids	−.78	+.99
Starch	−.79	+.98
Protein	−.93	+.99
Vitamin A value	+.89	−.69
Ascorbic acid	+.58	−.78

Significance
[1] ± .81 at 5% level and ± .92 at 1% level
[2] ± .55 at 5% level and ± .83 at 1% level

by total solids, A.I.S., starch, protein, or vitamin A value correlates highly with organoleptic quality.

Lee *(10)* in his studies of maturity of frozen lima beans found high correlation between organoleptic rating and the following: specific gravity, total solids, A.I.S., and texture meter readings. High correlations were also found between specific gravity and A.I.S. and between specific gravity and total solids. Kramer and Smith *(9)* correlated the quality with nutritive factors of canned lima beans. They found that A.I.S. (maturity) correlated well with moisture, protein, carbohydrates, calcium, and iron contents. They also found an excellent correlation between A.I.S. of canned beans and tenderometer readings.

Table 5 shows the results of the analyses of commercially frozen lima beans grown in different areas in California. The organoleptic score of Fordhook rated consistently higher than that of the baby limas. There are two possible explanations for this result: the judges may have preferred the Fordhook type; or the baby lima varieties need a longer cooking period to be of the same relative texture as the Fordhooks. The vine type of baby limas (Wilbur), usually grown for dry beans, also rated as high organoleptically as the bush garden type (Clark's Bush) when harvested at the green stage at aproximately the same A.I.S. value.

The mineral content of the baby type of lima beans, particularly the phosphorus content, was found to be higher than that of the Fordhooks. Except for total sugars there appears to be little difference between Clark's Bush and Wilbur in the constituents analyzed. Clark's Bush contained 0.7 g. total sugars, while Wilbur had an average of 1.4 g. total sugars per 100 g. of beans. Little difference was noted between U. S. Grade A and B Wilbur beans from Sutter County. Also there appears to be little difference in nutrient composition between U. S. 242 Fordhooks and the Concentrated Fordhook varieties. The U. S. 242 sample from Santa Clara County may have been less mature than the other Fordhooks analyzed since the total solids, A.I.S., starch, and protein values are lower and the sugar content higher. This sample rated highest in the organoleptic tests among the commercially frozen limas. No relationship can be noted between nutrient composition and the area in which the lima beans were grown.

Although the taste panel scored the Fordhook type of beans higher in texture, it is interesting that the nutrient value analyses showed in most constituents the baby type of lima beans to equal or surpass the Fordhooks for equivalent U. S. Grades.

SUMMARY

Few distinguishing differences were apparent among the varieties of beans studied. The most apparent differences occurred between the major bean types (baby lima and Fordhook) in organoleptic evaluation and mineral composition. Fordhook beans received higher organoleptic scores for cotyledon texture, whereas baby limas had generally a higher nutrient content.

Trends during maturation were marked. In both major bean types, maturation involved increase in the total solids, alcohol-insoluble solids, starch, protein, and mineral contents. On the other hand, total sugars,

TABLE 5

Commercial lima beans — based on 100 g. edible beans

California growing location	Variety	U. S. grade	Organoleptic score[1]	Total solids	Alcohol-insoluble solids	Total sugars	Starch	Protein	Fat	Ca	Fe	P	Carotene alpha	Carotene beta	Vita. A I.U.	Ascorbic acid	Thiamine	Riboflavin	Niacin
County			mean	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	*mg.*
Frozen Baby Type																			
Stanislaus	Clark's Bush	A	3.7	35.9	32.1	0.7	9.0	8.3	1.5	57	2.5	127	.04	.13	250	13	.06	.07	.9
Sutter	Clark's Bush	A	3.6	33.6	30.4	0.7	9.5	7.9	1.4	59	3.1	147	.05	.13	260	13	.11	.07	.7
Sutter	Wilbur	A	3.8	32.5	29.7	1.6	9.1	8.0	1.3	56	3.3	146	.04	.12	230	12	.10	.06	.9
Sutter	Wilbur	B	3.6	33.2	29.8	1.4	10.0	7.9	1.5	46	3.2	137	.03	.11	210	13	.12	.07	1.0
Sutter	Wilbur[2]	A	....	32.8	29.5	1.2	9.4	7.7	1.5	48	3.4	141	.05	.13	260	13	.09	.07	.9
Frozen Fordhook Type																			
Stanislaus	U. S. 242	A	5.6	28.0	24.0	1.7	9.3	6.3	1.1	34	1.9	88	.03	.10	190	15	.07	.06	1.2
Santa Clara	U. S. 242	A	6.2	23.7	18.7	2.3	6.5	5.4	1.3	34	2.5	88	.02	.13	230	17	.08	.07	1.0
Ventura	Conc. Fordhook	A	5.0	28.0	22.9	1.6	8.0	5.9	1.3	42	2.2	82	.02	.11	200	19	.07	.07	.9
Orange	Conc. Fordhook[3]	A	4.5	28.2	24.8	0.8	8.9	6.4	1.1	45	1.6	67	.04	.12	230	15	.07	.07	.9

[1] Measurement of texture on scale of 7 points: Score 7—smooth, moist; Score 1—dry, granular.
[2] Grade A Beans of the Wilbur variety were grown in Sutter County. Original sample was Grade B but by removal of the white beans the sample was classified Grade A.
[3] Partially thawed out when received from Orange County.

vitamins, and organoleptic scores decreased with seed ripeness. Differences due to locality grown were not noticeable in this investigation.

Blanching and freezing caused little change in the composition with respect to total solids, alcohol-insoluble solids, starch, proteins, and fats, but total sugars decreased. Ascorbic acid, vitamin A values, thiamine, riboflavin, and niacin were all lower as the result of processing.

Alcohol-insoluble solids and organoleptic scores were significantly correlated with each other and with the following: total solids, alcohol-insoluble solids, starch, protein, and vitamin A contents. Ascorbic acid was significantly correlated with alcohol-insoluble solids, but there was no significant relationship with the organoleptic scores.

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ON THE DETERMINATION OF ACETYL GROUPS IN PECTIC SUBSTANCES[a, b]

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Several factors have contributed to the present interest in the acetyl groups in pectic substances. The observations that some fruit pectins contain up to 3% of acetyl groups *(6)* and that acetyl groups inhibit the enzymic hydrolysis of the polygalacturonic acids *(12)* indicate that the acetyl groups in pectins might have more significance than previously suspected. The presence of acetyl groups in beet pectins is important on account of the influence of these groups on the jelly-forming ability *(9)*.

Although it has been known for some thirty years that acetyl groups occur in pectins *(4)*, until recently little dependable information was available on the true proportion of these groups. Unfortunately, the classical analytical methods of organic chemists give erroneously high results when applied to the determination of acetyl groups in pectins. In recent years Schneider and Boek *(10)*, Henglein and Vollmert *(3)*, Berglund *(2)* Pippen, McCready, and Owens *(8)*, Solms *(11)*, Arvidsson and Schiller-Meier *(1)*, and Owens *et al. (7)* described new methods or modifications of existing methods for the determination of acetyl groups in pectic substances. All these procedures employ treatment with alkali followed by distillation for the separation of the acetic acid formed upon saponification of the acetic acid ester. Many of these methods have been suspected of giving erroneous results at times.

The recent development of chromatographic techniques for the separation, identification, and determination of small quantities of organic acids seemed to offer possibilities as an aid in the selection of a most suitable method. The present paper reports some observations on the saponification of acetyl groups in pectins and on the possible formation of acetic and formic acids during the saponification. In addition, a simple, quick method which does not require distillation is described for the quantitative determination of the acetic acid in such hydrolyzates.

EXPERIMENTAL

Adequate saponification vs. destructive formation of acetic and formic acids. Acid hydrolysis of the acetyl groups in pectins is slow *(1)* and the temperature and acid required for complete deacetylation within a day or two are such that considerable destruction of the pectin and formation of volatile acids are likely to occur. We have made some preliminary tests to hydrolyze the ester by a commercial pectolytic enzyme (Pectinol 100 D). Only 6.4% of the acetyl groups were removed in one day and 14.9%

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in 3 days. Therefore, saponification with alkali seemed the only practical method which warrants consideration today. The main problem is to remove the acetyl groups completely without the formation of additional acetic and formic acids. It is in a search for such a balanced treatment that the technique of chromatographic separation and estimation of acetic and formic acids was found useful.

More concentrated pectin digests were needed with chromatography than with methods applying distillation. Evaporation of the alkali-treated digests after adjustment of the pH to 8.0 was first tried. As shown in Table 1, evaporation at 95°C. or in vacuum at room temperatures still produced both acetic and formic acids and therefore this approach was abandoned.

TABLE 1

Formation of acetic and formic acids in beet pectin and galacturonic acid solutions adjusted to pH 8.0 and evaporated at 95°C., or at 25°C. in vacuum

Sample	Acid in mg. per 100 mg. sample[2]			
	Evaporated at 95°		Evaporated at 25°	
	Acetic	Formic	Acetic	Formic
Beet pectin SBP[1]	0.68	0.78	0.93	1.64
Galacturonic acid	3.92	9.37	0.59	6.85

[1] Acetyl content 0.30%.
[2] The acid values given are the highest observed in a series of six determinations.

Digestion with a pectolytic enzyme before saponification was found helpful in making more concentrated solutions. With pectic substances containing about 1% acetyl groups, 1.0 g. of the sample when slurried in 10 ml. water and digested with 0.10 g. of Pectinol 100 D at 25-35°C. overnight eliminated the high viscosity which usually makes the handling of such concentrated pectin solutions difficult. Such pre-digestion also prevented the precipitation of pectic acid when the pH was lowered after the saponification. The digests were eventually made up to 25 ml. and thus in the present case we have dealt with a 4% solution. More concentrated solutions have been used with some samples containing a lower proportion of acetyl groups.

In acetyl group determinations on some partially acetylated pectic acid samples, the enzyme digestion was slow. This is in agreement with the observation of Solms and Deuel *(12)*. Fortunately, the higher yields of acetic acid from acetyl pectins allowed the use of small (0.2 g.) samples and in such instances the enzyme digestion can be omitted.

For the chromatographic determination of acetic and formic acids, the method described by Marvel and Rands *(5)* was used. In making up the column the silicic acid was slurried with chloroform containing 1% n-butanol (v/v). The sample (3 ml.), mixed with silicic acid was introduced as a powder on the top of the column and then slurried with some of the same solvent mixture *(13)*. Only 10% n-butanol in chloroform was used for development. One drop each of cresol red indicator and of a 2% solution of Dreft were used in the titration of the 10-ml. fractions with 0.02 *N* NaOH in 25-ml. glass-stoppered cylinders. The recovery of acetic and formic acids on the column averaged about 95%. The presence of 0.1 mg. of acetic or formic acid in the 3-ml. sample of digest could be clearly shown.

Saponification with 0.1 *N* NaOH has been often used for the removal of the acetyl groups of pectin *(1, 2, 8)*. Our results indicate that overnight treatment of 1.0 g. of pectin with 25 ml. of 0.1 *N* NaOH does not always give complete deacetylation. Of course, some of the alkali is consumed by neutralization and by the saponification of the methyl ester. In one experiment, using 1 g. of "Pectinum N.F." and 25 ml. of 0.1 *N* NaOH, the pH in 15 minutes dropped to 10.4 and in 3 hours to 9.5. According to the results in Table 2, only slow deacetylation can be expected at 9.5 pH and below. However, with cold 0.1 *N* NaOH the danger of formic acid production still exists. Whereas we have never found formic acid in a mixture of 1 g. of pectin kept in 25 ml. of 0.1 *N* NaOH at 25°C. overnight, it was found in one instance when the pectin was neutralized to pH 7 before the addition of the 25 ml. of alkali. Pure galacturonic acid treated in the same manner gave both acetic and formic acids.

TABLE 2

Removal of acetyl groups from beet pectin[1] with NaOH

Saponification at pH[2]	Per cent of total acetyl obtained after treatment for			
	0.5 hr.	1.0 hr.	2.0 hr.	3.0 hr.
9	.....	20.8	.....	.....
10	.....	50.8	.....	.....
11	64.0	84.9	99.4	100.0

[1] Acetyl content (calculated as CH_3CO) 3.22% as obtained by the procedure outlined later in this article.
[2] pH held constant by addition of alkali.

On the basis of these observations, it seemed desirable to use stronger alkali but a shorter period of saponification. When pectin solutions were digested with the enzyme, neutralized, and then allowed to stand in 0.2 *N* NaOH for 3 hours, maximum results were obtained but no formic acid could be found in the digests. Using 1 g. of pectin and 20 ml. of 0.2 *N* NaOH, the pH was still above 11 after 3 hours. Formic acid was not found after 5 hours of alkali treatment but usually was present after 24 hours. Some of the results obtained in such mixtures are shown in Figure 1. The amounts of acetic acid found for 3, 18, and 96 hours were similar (total titrations of 3.18, 3.02, and 3.00 ml. 0.02 *N* alkali, respectively), whereas the amount of formic acid increased with longer treatment.

Separation of acetic acid from the digest by liquid extraction. At first we tried to use the silicic acid column for the quantitative estimation of the acetic acid in the digest. However, such an approach saves little time in comparison with distillation and also requires constant attention. Therefore we have investigated the possibility of extracting the acetic acid from the digest by an immiscible solvent. The eluant used in chromatography (10% butanol in chloroform) gave satisfactory results. Diminishing the proportion of butanol in the chlorofrom decreased the effectiveness of the extraction.

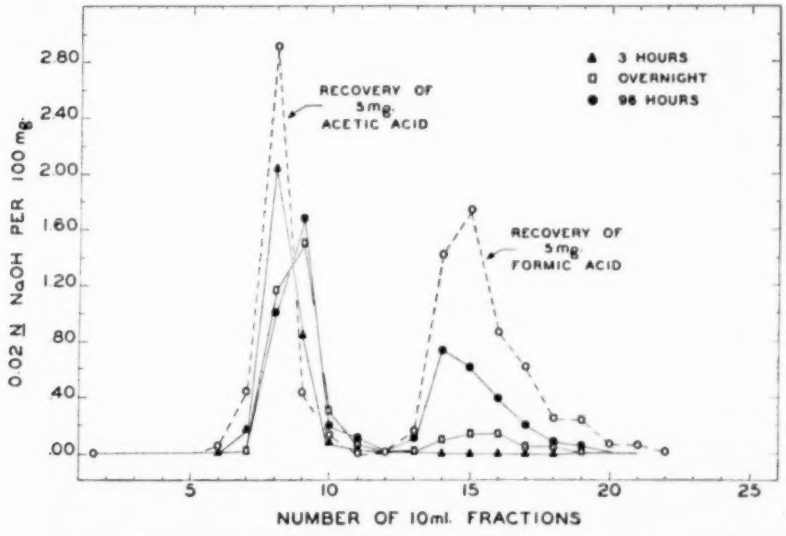

Figure 1. Chromatographic determination of acetic and formic acids in beet pectin held in 0.2 N NaOH at 25 C. Dotted lines indicate recovery of 5 mg. each of pure acetic and formic acids.

The partition ratio of acetic acid between water and the butanol-chloroform mixture at 20°C. was found to be 4.0:1. What is more important, galacturonic acid is for practical purposes completely insoluble in the organic phase. We have chosen 2 minutes of vigorous shaking of 2 ml. digest and 50 ml. of the butanol-chloroform mixture. Repeated tests indicated a recovery of 88.5% of the acetic acid from acetic acid in water or added to pectin digests. Therefore, the factor of 1.13 is used in the computations described below for estimating the acetic acid by titration of aliquots of the butanol-chloroform phase.

The extraction is not fully effective when the pH of the 2-ml. sample of the digest is above pH 3.0. At pH 3.5 the extraction gives results which are 20% lower whereas at pH 4.0 the proportion of acetic acid extracted is 30% lower than that obtained at pH 3.0. The extraction is equally effective at pH 2.0 and 3.0. Hence in the procedure outlined below the pH of the digest is adjusted to 2.0-2.5 before extraction with the butanol-chloroform mixture.

Suggested procedure for the determination of acetyl groups in pectic substances. On the basis of the observations reported above, the following procedure is suggested for the determination of acetyl groups in pectins without the application of heat and without distillation:

a. One g. of the pectic substance is digested at 25-35°C. overnight with 10% of its weight of Pectinol 100 D and 10 ml. water. If the acetyl content of the pectin is above 1%, a proportionately smaller, if it is below 1%, correspondingly larger sample should be used.

b. The enzyme digest is neutralized with 2 *N* NaOH, using a small piece of litmus paper for indicator. Then enough alkali and water are added to give a total volume of 20 ml. containing 0.2 *N* NaOH. This is allowed to stand for 3 hours at about 25°C. Afterwards the pH of the digest is adjusted to 2.0-2.5 with 5 *N* H_2SO_4, and the solution is made up to 25 ml. and mixed.

c. To 50 ml. chloroform containing 10% butanol measured into a separatory funnel, 2 ml. of the above digest is added and the mixture is vigorously shaken for 2 minutes. The mixture should be at 20°C. The two phases are allowed to separate, and about 45 ml. of the organic solvent mixture is slowly drained off into a dry graduated cylinder. Now 2 20-ml. aliquots of the butanol-chlorofrom mixture are titrated with 0.02 *N* NaOH using 1 drop each of 0.1% cresol red and 2% Dreft solutions.

d. First the mg. of acetyl groups (CH_3CO) found is calculated for the 50-ml. total of the organic solvent. Then the result is multiplied by the factor 1.13 to correct for the acetic acid in the water phase. Finally the acetyl content of the sample is calculated on the basis of its portion represented by the 2 ml. used in the determination. Duplicate extractions with duplicate titrations are suggested.

Some results obtained by this procedure are shown in Table 3.

TABLE 3

Acetyl contents of some pectic substances

Sample	Number of determinations	Acetyl as CH_3CO	
		Range %	Average %
Pectic acid[1]	2	0.10-0.13	0.12
Apple pectin	6	0.52-0.63	0.58
Citrus pectin, 150 grade	4	0.39 (all)	0.39
Citrus pectin NF	3	0.22 (all)	0.22
Swedish beet pectin	4	0.29-0.30	0.30
Beet pectin BP542	4	2.96-3.31	3.15
Acetyl pectin A	3	3.84-3.95	3.90
Acetyl pectin B	3	0.57-0.73	0.67

[1] Prepared from commercial citrus pectin by the method of H. H. Mottern and H. L. Cole [*J. Am. Chem. Soc.*, *61*, 2701 (1939)].

DISCUSSION

It is clear from the statements in the literature and from the data presented in this paper that care must be exercised in selecting the conditions for alkali treatment of pectic substances in the determination of their acetyl content. It would seem that while several of the methods now in use (*2, 8*) may well give the correct results at certain times, at others they may either provide incomplete saponification or lead to the formation of additional acids. Indeed, the balance between these two extremes is precarious with certain pectin preparations and one may well wonder if any one given set of conditions can give the correct results in all instances.

Silicic acid column chromatography is a suitable method for showing the presence of small quantities of formic and acetic acids in the digests. Our tests with galacturonic acid and pectins indicate that upon excessive treatment with alkali, formic acid appears in the digest sooner and in larger quantities than does the acetic acid presumably not derived from acetyl groups (Figure 1 and Table 1). The absence of formic acid in a saponified digest might be taken as an indication that the alkali treatment was not excessive. In other words, the concentration of the alkali and the length of saponification treatment should be extended until there is no further increase in the acetic acid yet no formic acid is produced. These criteria should be used both in modifying the conditions described here and in applying the alkali treatment to different types of pectins.

We have no illusions that the present method of quick determination of the acetic acid will replace the more exact albeit more time-consuming distillation procedures. Yet, it satisfies the requirements for a simple method not requiring elaborate equipment and suitable for serial determinations.

SUMMARY

Silicic acid column chromatography, applied to a study of the determination of the acetyl groups of pectins, was found useful in establishing the saponification treatment which will give complete removal of the acetyl groups. The absence of formic acid can be taken as an indication that the conditions of saponification were not unduly severe. A 3-hour treatment in 0.2 *N* NaOH at 20-25°C. gave satisfactory results with the pectin preparations tested during the course of these investigations.

A simple, quick method is described for the determination of acetic acid in pectin digests by extraction with an immiscible solvent. The results obtained on 8 pectin preparations are given to illustrate the utility of the method.

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SOURCES AND DETECTION OF VOGES-PROSKAUER REACTANTS IN CALIFORNIA VALENCIA ORANGE JUICE[a]

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The utility of the Voges-Proskauer *(17)* reaction in detecting bacterial metabolites responsible for specific off-flavors in frozen concentrated Florida orange juice has been demonstrated by Hill, Wenzel, and Barreto *(7)* and Byer *(3)*. This report is concerned with the application of their method to California Valencia orange juice, particularly as compositional differences may affect evaluation of data from different citrus producing areas.

The Voges-Proskauer reaction was selected by the original citrus workers because of its sensitivity for the oxidation-reduction compounds diacetyl and acetoin (acetylmethylcarbinol). Several methods are known for the detection of diacetyl. One of these, employing hydroxylamine, yields dimethylglyoxime, which subsequently condenses with urea to give a yellow compound *(19)*. The method does not appear to be sensitive below 15 p.p.m.

Other workers *(12, 18, 11, 13)* have reacted the dimethylglyoxime with a nickel salt to produce soluble tetravalent nickel dimethylglyoxime having a red color. The method, used on beer, appears to be sensitive at 0.2 p.p.m.

The hydroxylamine reaction, although highly specific for diacetyl, is time-consuming and requires meticulous technique. Other methods based on reaction with chromotropic acid or analine extraction *(15)* are either not specific or sufficiently sensitive.

The Voges-Proskauer reaction as modified by Barritt *(1)* results from the reaction of diacetyl and peptone with α napthol to produce a red color. The test also determines 2,3 butanediol (2,3 butylene glycol). The detectable limits appear to be 0.05 p.p.m. diacetyl, 0.5 p.p.m. acetylmethylcarbinol, and 40,000 p.p.m. 2,3 butylene glycol.

The Voges-Proskauer reaction, hereinafter referred to as the V-P reaction, although highly specific for the above three named substances, can be affected by other compounds, some of which are naturally occurring or degradation products in orange juice. The Byer modification *(3)* for citrus juices affords concentration of the significant materials to an easily detectable level and removal of same from a substrate containing a heterogeneity of interfering compounds.

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EXPERIMENTAL PROCEDURE

Organoleptic method. Organoleptic studies *(4)* were conducted to determine the sensitivity requirements of the method for California juice. The rejection threshold was determined by adding diacetyl (2,3 butanedione) to acceptable juice. The samples were submitted to a taste panel, employing conventional triangular and single sample techniques.

A statistically significant threshold was apparent at 0.25 p.p.m. diacetyl. The low threshold might be one of discrimination rather than identification. Several tasters found identification imposible below 0.5 p.p.m., although discrimination was possible at 0.25 p.p.m. The lower level had a foreign flavor described by them as "cardboard."

Chemical Method A. *Distillation method.* The earlier cited work of Hill *et al.* supported by the modification of Byer appeared to be the logical origin for studies on California juice. The latter technique utilizes distillation to remove diacetyl and acetoin from the highly colored juice, permitting accurate evaluation of color development in the distillate, and at concentrations above the sensitivity limit. The distillate readings can be utilized *per se* or calculated to a single strength juice basis by applying the concentration factor. Exploratory studies by the present authors, using the established distillation procedure and subsequent colorimetric manipulations, suggested further modifications.

Preliminary studies resulted in several alterations of the Byer method *(3)*: use of a 250-ml. sample with recovery of the first 10% distillate, dilution of same with an equal volume of water, and use of a 25-ml. aliquot of the mixture for the V-P reaction. The reacted products were then diluted one-half prior to reading. The dilutions were made to produce a curve whose slope would be significant when dealing with very small concentrations. The method:

Reagents:

2 *N* $KMnO_4$	Dissolve 64 g. $KMnO_4$, tech. grade in 1000 ml. distilled water. Boil soln. 1 hour and allow to stand overnight. Filter through Whatman No. 4 paper.
Anti-foam.	Dow Corning Antifoam A.
Alpha napthol solution.	Dissolve 5 g. α napthol (Eastman No. 170) in 95 g. 99.6% isopropanol.
KOH-creatine solution.	Dissolve 2 g. creatine (Eastman No. 951) in 98 g. 40% potassium hydroxide solution.

Equipment:

Spectrophotometer:	Coleman Model 6A or equivalent.
Boiling Flasks:	1000-ml. round-bottom, short-ring neck flasks.
Condenser:	Westphal 300 mm.
Delivery Tubing:	9 mm. i.d.
Cylinders:	50-ml. mixing, graduated, with glass stoppers.

Procedure:

Place 250 ml. single strength or 12° Brix reconstituted orange juice in 1 liter boiling flask. Add 10 ml. 2*N* – $KMnO_4$ and a drop of anti-foam. Shake and allow to stand 5 minutes. Distill into a 50-ml. mixing graduated cylinder at a rate to recover 25 ml. in 10 minutes after coming to a boil. Dilute to 50 ml. with distilled water. Shake and remove 25 ml. with a pipette. Add 10 ml. α napthol reagent and 4 ml. KOH-creatine solution. Mix by inverting the cylinder for exactly 1 minute. Pipette 5 ml. of the reacted mixture into a spectrophotometer cuvette containing 5 ml. distilled water. Shake and read at 550 mμ with the instrument set at 90% transmission on a reagent blank consisting of 25 ml. distilled water, 10 ml. α napthol and 4 ml. KOH-creatine solution after a reaction time of 1 minute. This reading corresponds with

the diacetyl content. The acetylmethylcarbinol content can be estimated by allowing the reaction mixture to stand for 30 minutes. A reagent blank corresponding to the same time should be employed. The difference in the 1- and 30-minute readings represents the acetylmethylcarbinol content.

The authors elected to prepare a standard curve which would read concentration of diacetyl direct, rather than employ a conversion calculation. A study of curves prepared by adding known quantities of diacetyl to distilled water and 12° Brix sucrose solution respectively, and distilling same according to the method of Byer *(5)* indicated that

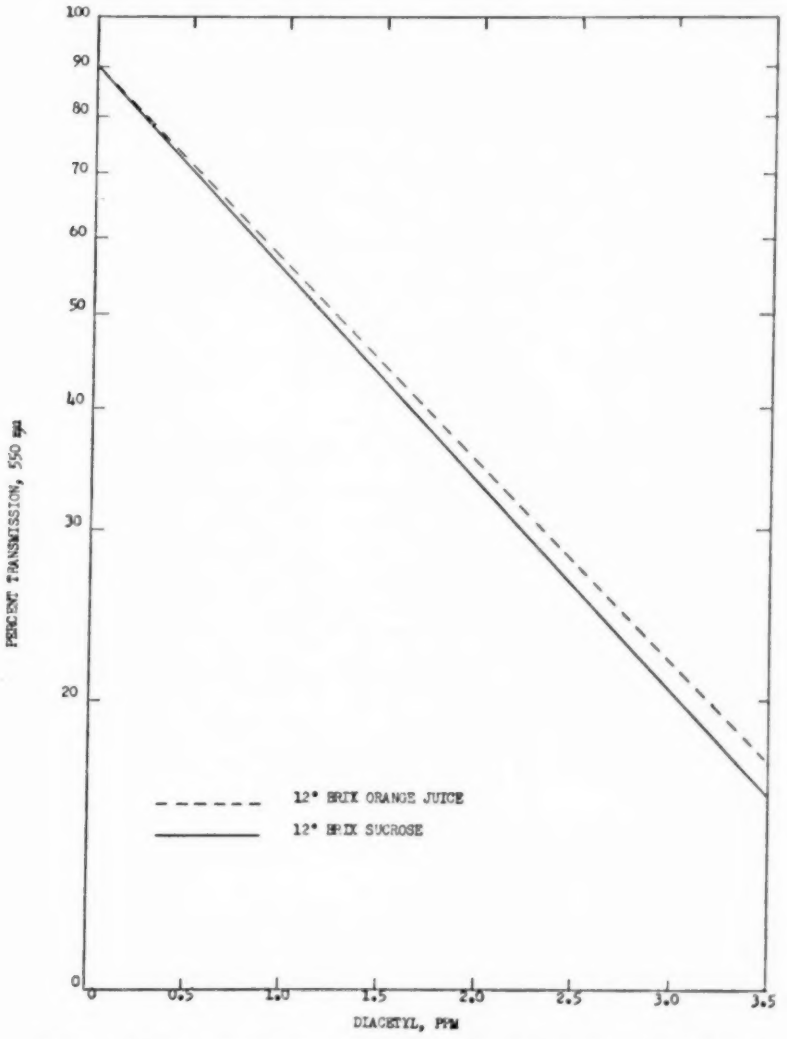

Figure 1. Diacetyl concentration in 12° Brix sucrose and orange juice solutions.

recoveries from the sucrose solution were of a lower order due to the per cent of dissolved solids present. Since this per cent of soluble solids would obtain in an orange juice sample, it was decided to eliminate the water calibration curve and use the sucrose curve as a reference. The curve obtained at 550 mμ is illustrated in the solid line of Figure 1.

When diacetyl was added to samples of reconstituted orange juice (12° Brix) the recoveries referred to the sucrose curve were of a very low order, i.e., 60-65%. Repeated tests led to the conclusion that the reducing substances naturally present in orange juice were responsible for promoting an equilibrium shift in the direction of acetylmethylcarbinol and 2, 3 butanediol. Several oxidizing agents were tried. The addition of 2*N*-$KMnO_4$ in an amount sufficient to destroy the ascorbic acid present brought recoveries up to 95-98%. The juice curve obtained is illustrated as the broken line in Figure 1.

Chemical Method B. *Inert Gas Stripping Method.* This procedure was developed as an alternate to the distillation method. It alleviates the disadvantages of the distillation method pointed out under *Results and Discussion*, where the inert gas stripping procedure is described in detail.

Bacteriological method. Bacteriological samples were obtained at the sources noted under *Sampling Procedure*. Samples collected on "swing" and "graveyard" shifts were held at 8°-10°C. prior to plating on the succeeding day. The material was diluted 1:1000 with sterile distilled water and plated on orange serum agar at pH 5.45 *(16)*. After incubation for 48 hours at 30°C. (86°F.) total population was recorded and selected colonies were fished from each plate and transferred to orange serum agar slants. The slants were incubated until good growth was apparent. The selection of bacteria and yeast colonies was carried out in a manner to include both indigenous and atypical types. The slants were submitted to the Food Technology Department of the University of California (Davis) for identification and extended biochemical study.

The cultures received at the University of California were purified by restreaking on orange serum agar. After purification, the cultures were identified by the use of commonly accepted methods *(2, 5)*.

The morphological characteristics of the microorganisms were determined by the use of the Gram stain and the Fisher and Conn *(6)* flagella stain, as well as noting cell size and shape under the phase microscope. The catalase negative lactic acid bacteria were separated from the catalase positive types by determination of the ability to produce the enzyme on orange serum agar. The non-volatile acid end-products of glucose metabolism (i.e., lactic acid or gluconic acid) were determined by paper chromatography *(10)*. Gas and acid production from glucose and lactose and ethanol oxidation, were used to separate the catalase positive, gram negative bacteria into their respective genera. The genera of yeasts were identified by commonly accepted methods *(9)*.

After generic identification of the cultures, they were subjected to screening for tolerance to different pH values in filtered orange juice. Those cultures which grew in filtered orange juice in the pH range of 3.5 to 4.0 were tested for their ability to grow and produce V-P reactants in the same menstrum at pH 4.0.

The ability of the cultures to produce V-P reactants in the five major fermentable substrates of orange juice (glucose, fructose, sucrose: malic and citric acids) was tested in a peptone yeast extract broth adjusted to pH 4.0.

Sampling procedure. The following plant stations were selected for bacteriological and chemical checks:

a. Raw juice supply tank to evaporators.
b. Second effect of evaporators (20° Brix).
c. First effect of evaporators (58° Brix).
d. Cutback juice tanks.

Only one evaporator was studied per operating cycle, i.e., clean-up to clean-up. Samples were taken at each station, three times each 24 hours. Samples were procured in duplicate, one for bacteriological work and the other for chemical study. The chemical samples were frozen and the bacteriological samples were stored at 8°-10°C.

In addition to the processing line checks, periodic samples of juice obtained from fruit classified according to specific defects were examined chemically and bacteriologically. Juice from fruit judged to be of acceptable to superior quality was also evaluated. Such fruit was obtained directly off the tree or upon arrival at the packing house. The

fruit defects selected for study: *(a) splits, (b) rots, (c) internally damaged (crushed), (d) shrivelled, (e) anaerobic.*

The classification *anaerobic* refers to fruit that has respired in a limited atmosphere. An example would be fruit stored for a prolonged period in the center of a load in a truck or fruit bin. This condition can be duplicated by allowing fruit to respire 72 hours in a closed container.

RESULTS AND DISCUSSION

A. Chemical phase—distillation. Development of the modified distillation procedure was followed by an examination of *sound* and *defective* fruit to ascertain the range of V-P reactants to be found therein. Use of an oxidizing agent in the substrate precluded reading natural diacetyl *per se;* however, supplemental studies on juice containing added acetoin failed to demonstrate a significant increase in the reading. Table 1 illustrates the results obtained on juice from sound, acceptable fruit; each sample represents a portion of the juice from 20 pounds of fruit.

TABLE 1

Voges-Proskauer reactants (as diacetyl) in juice from acceptable fruit

Date	P.P.M. diacetyl	Date	P.P.M. diacetyl
9-17-53	0.78	10-21-53	0.61
9-23-53	0.55	10-29-53	0.69
9-30-53	0.68	11-4-53	0.64
10-7-53	0.85	11-5-53[2]	0.46
10-7-53[1]	0.56	11-12-53	0.93
10-14-53	1.05		

[1] Fruit obtained from tree.
[2] Fruit obtained at packing house. All others received at processing plant.

Data in Table 1 present an anomaly, in that the values are much higher than the rejection threshold of a taste panel, and (more than likely) of consumer acceptance. Control checks employing juice to which no potassium permanganate was added produced expected lower values which, however, were still far in excess of the taste threshold. These observations do not agree with those of Hill *et al. (7)* on Florida orange juice, in which the range of diacetyl was found to be none to 0.1 p.p.m. The nature of this variable juice blank was studied in detail and is reported later in this paper.

Further, an examination of the data shows no apparent trend which would allow the establishment of a correction factor. There also appears to be no correlation between time of season and concentration of V-P reactants.

TABLE 2

Voges-Proskauer reactants (as diacetyl) in juice from defective fruit

Defect	Date (1953)								
	9-17	9-23	9-30	10-7	10-14	10-21	10-29	11-4	11-12
	P.P.M. Diacetyl								
Splits	>3.5	1.07	1.15	1.19	1.96	1.51	0.81	0.91	1.76
Rots	>3.5	>3.5	2.28	1.63	>3.5	2.82	2.06	1.82	>3.5
Shrivelled	1.42	0.84	1.00	1.27	1.79	1.44	1.30	0.85	1.35
Internal damage	1.15	0.82	1.13	1.18	1.28	0.74	0.77	0.78	1.11
Anaerobic	0.84	0.67	0.60	1.08	1.52	0.87	0.70	0.84	0.91

Table 2 lists the values obtained by the distillation method on juice from fruit classified for specific defects.

Periodic bacteriological examination of the juice from defective fruit showed a predominant microflora of *Lactobacillus* and *Leuconostoc* in all classifications except *internal damage* and *anaerobic*. The latter defects contained a yeast microflora, although a few bacterial isolates were found. The V-P reactants conformed to the bacteriological findings, with the highest values occurring in rotten fruit, followed by splits.

The trend in V-P reactant concentration during an operating cycle was developed by 8-hour checks at 4 sampling points. The duration of checks was from evaporator clean-up to shutdown. Bacteriological checks on duplicate samples failed to establish a well-defined correlation, either in terms of total count or incidence of specific genera of bacteria. However, several operating cycles did reveal significant increases in *Lactobacillus* and *Leuconostoc* at the 20° Brix stage of concentration that correlated with the V-P test. These instances were in the minority, however.

Data accumulated during the chemical phase of the study demonstrated the utility of the distillation method in delineating trends. However, single sample values were not reliable because of the highly variable concentration of "non-significant" reactants, i.e., other than acetoin or diacetyl. Citric acid, ascorbic acid, pectin, and orange oil were investigated by adding each to 12° Brix sucrose and distilling. The distillate gave a negative V-P reaction in each instance. The isopropanol employed in the alpha napthol reagent was checked against absolute ethanol with no discrepancy noted.

Attempts at elimination of the interfering substances included treatment of the substrate with ferric sulfate-ferric chloride solution, 10*N* sulfuric acid digestion, and neutralization of the sample. All were to no avail; in fact, neutralization of the sample tripled the reading on the distillate.

Juice from fruit picked off the trees and brought direct to the laboratory, and juice extracted from plant receipts, was selected for chromatographic study; 1500 ml. of each was distilled at a rate to recover 150 ml. in 1 hour. Duplicate juices containing 1 p.p.m. added diacetyl were also run. The distillates were examined by the Voges-Proskauer method and silica strip chromatography *(8)* using re-purified diacetyl and acetoin as controls. The chromatographic examination revealed the constant presence of an unidentified substance.

This substance was discernible in distillate from juice that had been distilled 1 hour. Twenty-five ml. aliquots showed the substance to distill at a uniform rate. Later work in the bacteriological phase of study suggested that this substance may be a product of sugar degradation, since heated solutions of sucrose, fructose, or glucose at pH 3.5 gave a qualitative V-P test.

B. Chemical phase. *Inert Gas Stripping.* The volatility of diacetyl in comparison with that of acetoin suggested that a pure recovery of the former could be made, provided the interfering substances did not appear within the boiling range of the two. Inert gas stripping was conducted in the apparatus shown in Figure 2. Preliminary examination showed that diacetyl could be quantitatively recovered from water and 12° Brix sucrose.

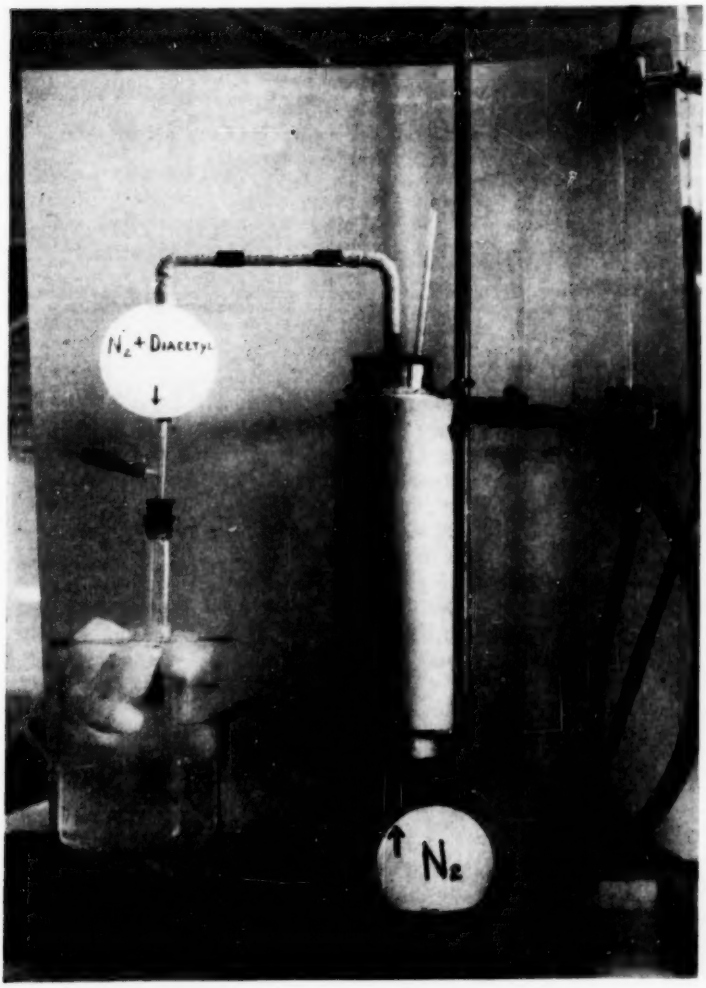

Figure 2. Inert gas stripping apparatus.

A standard curve was prepared and the study expanded to include orange juice.

The method:

Reagents:

Same as for distillation method, except that the potassium permanganate is eliminated.

Equipment:

Stripping column. Stainless steel, 6 cm. inside diameter, 30 cm. length. Equipped with steam jacket for heating. Nitrogen supply. Pressure regulator and flow meter. Flow 700 ml./min.
Diffuser. Stainless steel. 6 cm. diameter. Medium porosity. Gas scrubber tube. Fritted glass. 1.2 cm. outside diameter.
3-5 cm. length.
Ice bath.
Steam supply.
Graduates. 50-ml. glass stoppered.
Thermometers.

Procedure:

Assemble apparatus as shown in Figure 4. Adjust ice bath to 0°C. Place graduate containing 25 ml. distilled water in ice bath. Pour 250 ml. juice into column and connect tower discharge to gas scrubber tube in ice bath. Turn steam on jacket of column until temperature of juice reaches 90°C. Start flow of nitrogen gas and stop watch. Meter nitrogen at a rate of 700 ml. per minute. The juice temperature will drop to about 85°C. after flow of nitrogen is started. Allow temperature to rise again to 90°C. and cut off steam. Continue stripping for a total of 10 minutes. Rinse gas scrubber three times into graduate. Dilute the collected material to 50 ml. and use a 25-ml. aliquot for the V-P reaction. Proceed as described for the distillation method.

TABLE 3

Voges-Proskauer reactants (as diacetyl) in orange juice

Source	Method	
	Distillation	Gas stripping
I	*P.P.M.*	*P.P.M.*
12° Brix Orange Juice	0.45	0.03
I plus 0.5 p.p.m. Diacetyl	1.11	0.50
II		
12° Brix Orange Juice	0.55	0.05
II plus 0.5 p.p.m. Diacetyl	1.09	0.55

The utility of the method is illustrated in Table 3, in which samples of orange juice with and without added diacetyl are compared by the distillation and inert gas stripping techniques.

It is of interest to note that the V-P values on untreated juice (gas stripped) approximate the values for natural juice found by the Florida workers *(7)*, i.e., none to 0.1 p.p.m. The method permits the direct recovery of diacetyl from California Valencia orange juice without the inclusion of interfering substances found in the distillation method. Additional studies with acetylmethylcarbinol in the substrate do not show any appreciable recovery of that substance.

BACTERIOLOGICAL PHASE

The 120 cultures received at the University of California, Davis, were separated into 67 cultures of *Lactobacillus*, 13 cultures of *Leuconostoc*, 1 culture of *Pediococcus*, 20 cultures of *Acetobacter*, 6 cultures of yeasts of the genera *Candida*, *Kloeckera*, and *Torulopsis*, and 13 other catalase positive, gram negative cultures. The origin and widespread occurrence of these types are shown in Table 4.

TABLE 4

Generic classification of 120 cultures isolated from orange juice on orange serum agar by E.O.P. Company

Sources	Number of Isolates	Classification				
		Lactobacillus	*Leuconostoc*	*Acetobacter*	Yeast	Other
		Number of cultures in each genus				
Production Samples:						
Pan Feed Tanks	24	10	5	5	0	4
Cut-Back Tanks	18	4	1	8	1	4
Evaporator 2nd Effect 20° Brix	27	22	2	0	1	2
Evaporator 1st Effect 58° Brix	22	16	4	0	0	2
Defective fruit samples:						
Shrivelled	7	3	0	2	1	1[1]
Internal Damage (Crushed)	5	1	1	2	0	1
Splits	5	2	0	1	2	0
Rots (Soft)	4	2	0	2	0	0
Anaerobic[4]	5	5	0	0	0	0
Control	3	2	0	0	1	0
Totals	120	67	13	20	6[3]	14[2]

[1] *Pediococcus.*
[2] Thirteen remain unidentified.
[3] Three genera: *Candida*, *Kloeckera*, and *Torulopsis*.
[4] Internally damaged fruit which has been stored in a limited atmosphere.

Production of V-P reactants in orange serum. After generic identification of the cultures, they were subjected to screening for tolerance to different pH values in filtered orange juice. Those cultures which grew in filtered orange juice in the pH range of 3.5 to 4.0 were tested for their ability to grow and produce V-P reactants in the same menstrum at pH 4.0. The results of this experiment are shown in Table 5. It will be noted that the cultures of *Lactobacillus* and *Leuconostoc* produced significant positive V-P reactions after two days' incubation at room temperature (25°C.). It is particularly interesting to note that the yeasts also produced a significant number of positive reactions after 3 days' incubation. It is obvious from these results that the production of V-P reactants in filtered orange juice is not confined to any one genus, but rather to cultures of any of the genera found capable of growing in the filtered juice at pH 4.0.

Production of positive V-P reactants in different substrates. The 5 major fermentable substrates in orange juice include glucose, fructose, sucrose, and malic and citric acids. The ability of the cultures to produce

TABLE 5

Positive V-P reactions after 1 to 7 days in orange serum

Genera	Number of Isolates	Number of positive V.-P. reactions after incubation at 25°C. (77°F.) for					
		1 day	2 days	3 days	4 days	5 days	7 days
Lactobacillus	66	15	40	35	38	45	41
Leuconostoc	13	3	13	12	12	12	13
Acetobacter	10	0	0	0	1	1	3
Yeast[a]	39	0	4	10	22	24	23
Others	14	0	0	0	2	5	5

[a] Includes 33 yeasts from Recca and Mrak (*14*).

V-P reactants in these different substrates was tested in a peptone, yeast extract broth adjusted to pH 4.0. As shown in Table 6 positive V-P reactions were produced from the various substrates. It may be concluded, therefore, that any of the major fermentable constituents of citrus juice may give rise to V-P reactants through the activity of the various groups of microorganisms.

TABLE 6

Positive V-P reaction in different substrates pH of 4.0 in peptone yeast extract broth[1]

Genera	Number of Isolates	Number of positive V.-P. reactions from[3]				
		Glucose	Fructose	Sucrose	Malic acid	Citric acid
Lactobacillus	66	25	30	23	24	40
Leuconostoc	13	0	4	1	1	6
Acetobacter	10	6	8	5	5	4
Yeast[2]	39	14	14	6	1	0
Others	14	8	8	8	8	8

[1] Basal medium composed of 0.5% Bacto peptone and 0.5% Bacto yeast extract in distilled water. The test substrate was used in a 1.0% concentration.
[2] Includes 33 yeast cultures from Recca and Mrak (*14*).
[3] After incubation for four days at 25°C. (77°F.).

SUMMARY AND CONCLUSIONS

The examination of distilled orange juice by the Voges-Proskauer reaction will produce usable trend curves for process control. However, California Valencia orange juice contains substances which produce non-significant values in excess of organoleptic standards, which preclude use of this method for single sample evaluation.

The utility of the Voges-Proskauer technique can be increased by inert gas stripping of the sample, permitting evaluation of single substances without "non-significant" interference.

Voges-Proskauer reactants can be produced by any of the genera of bacteria found capable of growing in filtered orange juice at pH 4.0. Any of the major fermentable constituents of citrus juice may give rise to V-P reactants through the activity of the various groups of microorganisms.

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ERRATA 1954

FOOD RESEARCH, 19 (1), p. 122, paragraph 5, line 3, read "none" for "some"; p. 128, paragraph 1, line 5, read "D" for "B"; p. 131, paragraph 1, line 2, read "8" instead of "3."

FOOD RESEARCH, 19 (1), p. 135, Table 1 and p. 136, Table 2 read "micrograms per 100 grams" for "Milligrams per 100 grams." The values refer to folic and biotin contents of germinated pulses.

DIFCO

Media for

MYCOLOGY

DIAGNOSTIC

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:

Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

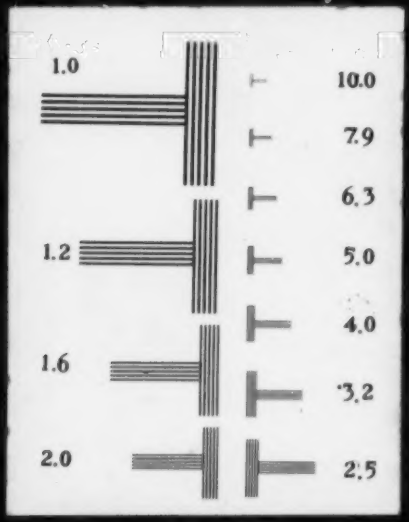
. . . and nutritional studies of fungi:

Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION,*
including descriptions of these media and their use,
is available on request.

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

RESOLUTION CHART



100 MILLIMETERS

INSTRUCTIONS Resolution is expressed in terms of the lines per millimeter recorded by a particular film under specified conditions. Numerals in chart indicate the number of lines per millimeter in adjacent "T-shaped" groupings.

In microfilming, it is necessary to determine the reduction ratio and multiply the number of lines in the chart by this value to find the number of lines recorded by the film. As an aid in determining the reduction ratio, the line above is 100 millimeters in length. Measuring this line in the film image and dividing the length into 100 gives the reduction ratio. Example: the line is 20 mm. long in the film image, and $100/20 = 5$.

Examine "T-shaped" line groupings in the film with microscope, and note the number adjacent to finest lines recorded sharply and distinctly. Multiply this number by the reduction factor to obtain resolving power in lines per millimeter. Example: 7.9 group of lines is clearly recorded while lines in the 10.0 group are not distinctly separated. Reduction ratio is 5, and $7.9 \times 5 = 39.5$ lines per millimeter recorded satisfactorily. $10.0 \times 5 = 50$ lines per millimeter which are not recorded satisfactorily. Under the particular conditions, maximum resolution is between 39.5 and 50 lines per millimeter.

Resolution, as measured on the film, is a test of the entire photographic system, including lens, exposure, processing, and other factors. These rarely utilize maximum resolution of the film. Vibrations during exposure, lack of critical focus, and exposures yielding very dense negatives are to be avoided.